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Effect of astaxanthin and cholesterol on growth, survival, and pigmentation of adult spiny lobster, *Panulirus ornatus* (Decapoda, Palinuridae)

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Abstract. Prior research on the spiny lobster *Panulirus ornatus* (Fabricius, 1798) determined that intermediate levels of cholesterol are important in diets, but astaxanthin may not be. Here we examined how the growth, survival and coloration of spiny lobster were influenced by the inclusion of both astaxanthin (50, 60 and 70 mg.kg^{-1}) and cholesterol (0.3, 0.4 and 0.5 %) in a two factor experiment. Overall, survival was 84.8% with no significant difference among dietary treatments. Lobsters grew the best when fed the diet containing the lowest cholesterol and greatest astaxanthin of the levels presented. The results of this study point out the need to examine the effects of dietary component addition across a range of inclusion levels simultaneously for multiple nutrients.
Key Words: astaxanthin, cholesterol, color, lobster, nutrition, specific growth rate.

Tóm tắt: Nghiên cứu trước đây trên tôm hùm bông *Panulirus ornatus* đã xác định cholesterol có vai trò quan trọng, tuy nhiên, astaxanthin thì chưa rõ ràng. Ở đây, thí nghiệm hai nhân tố được thực hiện để khảo sát ảnh hưởng của việc bổ sung hàm lượng astaxanthin (50, 60 và 70 mg.kg^{-1}) và cholesterol (0.3, 0.4 và 0.5%) vào thức ăn viên đến sinh trưởng, tỷ lệ sống và màu sắc của tôm hùm bông. Nhìn chung, tỷ lệ sống đạt 84.8% nhưng khác biệt không có ý nghĩa thống kê giữa các nghiệm thức cho ăn. Tôm hùm đạt tăng trưởng nhanh nhất trong khẩu phần ăn có chứa hàm lượng cholesterol thấp nhất và astaxanthin nhiều nhất trong các nghiệm thức thí nghiệm. Kết quả của nghiên cứu này chỉ ra rằng cần phải nghiên cứu ảnh hưởng đồng thời các thành phần bổ sung của nhiều chất dinh dưỡng khác nhau.
Từ khóa: astaxanthin, cholesterol, màu sắc, tôm hùm, dinh dưỡng, tăng trưởng riêng.

Introduction. The tropical spiny lobsters (Decapoda, Palinuridae) are one of the world's most valuable seafoods with great market appeal in Asia, Europe and America (Williams 2007). The lobster industry has a great potential as an aquaculture commodity in Viet Nam. In recent years, production of lobster has rapidly increased significantly contributing to the social and economic development in coastal rural communities. Inspired by this success, in 2001-02, more than 49,000 cages producing about 1,900 tonnes and having an export value of approximately US$ 70-75 million (Thuy & Ngoc 2004) were established in the central region of Viet Nam (Hung & Tuan 2009). Production is currently estimated to exceed 3,000 tonnes with an export value of US$ 90 million (Williams 2007).

Appropriate quantities of dietary cholesterol and astaxanthin are known to promote growth and survival of a range of marine crustaceans (Kanazawa 2001; Pan & Chien 2004; Niu et al 2009). In addition, cholesterol is an important precursor of bile acids, steroid hormones, molting hormones, and vitamin D (Kanazawa 2001). Dietary supplementation of astaxanthin has been proven highly effective in enhancing pigmentation, antioxidant status, and resistance to be various environmental stresses of crustaceans (Chien & Jeng 1992; Chien et al 2003; Tlusty & Hyland 2005; Barclay et al 2006; Niu et al 2009). However, lobsters, like other crustaceans have been found to be incapable of synthesizing both astaxanthin and cholesterol *de novo* (Meyers & Latscha 1997; Teshima & Kanazawa 1971). The optimum level of dietary cholesterol for lobster diets ranges from 0.12–0.5 %, depending on species, age and physiological condition (D’Abramo et al 1984; Kean et al 1985; Kanazawa 2001; Irvin & Williams 2009). The lobster's astaxanthin requirement to promote optimal weight gain and survival is approximately 50 mg astaxanthin per kg diet (Kristiansen et al 2004; Tlusty & Hyland 2005), but the amount required to maintain color similar to that of wild-caught adults is greater (Tlusty & Hyland 2005; Barclay et al 2006; Crear et al 2002).

Even though, both astaxanthin and cholesterol have been shown to be an essential dietary nutrient for crustaceans, little is known about the requirements of these nutrients for spiny lobsters. In addition, optimizing the amount of these nutrients is beneficial because they are expensive dietary ingredients. The cost of astaxanthin about US$ 3,000 kg^{-1} of an active ingredient (Barclay et al 2006), whereas the addition of 2 kg of cholesterol to 1,000 kg of feed represents more than 10 % of the total ingredient cost of the diet (Smith et al 2001). Therefore, the purpose of this study was to determine the optimal dietary astaxanthin and cholesterol levels to promote good growth and survival in adult spiny lobster *P. ornatus* under outdoor cage conditions.

Material and Methods

Experiment management. The spiny lobsters were hand-collected from the central coast of Viet Nam (11°31′00.43″ N; 109°15′40.22″ E) with an average weight of 243.4±50.06 gram. The lobsters were acclimated to the experimental conditions for one week prior to the start of an eight week feeding trial. Both the astaxanthin and cholesterol diet components had three levels, and these were included into diets in a two-factor design with three replicates per treatment. Lobsters were randomly distributed into 27 experimental cages with 10 adults per cages. All the groups were housed in (2m L × 2m W × 4m H) cages made from blue screen net. The lobsters were handed-fed to excess once daily at 17.00 h at approximately 5 % body weight. Every morning, feed residue, fecal waste, molted exuviae and other waste material were removed. An additional cage (4m L x 4m W x 6m H) held 30 lobsters which were fed a trash fish diet consisting primarily of *Portunus spp.* (swimming crabs). While this cage was not statistically considered in the treatment comparisons, it was used graphically as a reference point to compare the performance of lobsters fed the experimental diets to lobsters being reared under more commercial conditions.

Diet preparation and sample collection. Diets were fed in a two factorial experimental design with three dietary astaxanthin levels (50, 60 and 70 mg kg^{-1}) and three cholesterol levels (0.3, 0.4 and 0.5 %). The diets were formulated to provide a feasible range of astaxanthin and cholesterol for use within the spiny lobster culture industry in Viet Nam. Feeds were made at the Fish Nutrition Laboratory, Faculty of Aquaculture, Nha Trang University. The standard feed for this study was based on results of previous work by Hung (2010) and elsewhere (Smith et al 2003). The composition of the nine feeds is provided in Table 1. All formulated diets contained 58 % crude protein and 11 % crude lipid. The ingredients were mixed thoroughly, then adequate water was added and mixed until a dough was formed. The dough was extruded through a 5 mm die plate of the mincer (Chufood CS200 dough mixer, Chuseng Food Machinery Works Co. Ltd, Taiwan), which pressed the feed to make spaghetti-like strands. All experimental diets were dried in the forced air oven at 45°C for 12 h. The feed was then pelleted to a 2–3 cm length and stored until feeding at -20°C to avoid oxidation of the pigments.

At the end of the 8 weeks of feeding, all of lobsters in each cage were censused to determine survival, weighed to establish growth, and photographed to determine pigmentation. From these data, and the known amounts of food delivered per cage, the food conversion ratio per cage was calculated. Growth was expressed as specific growth rate (SGR) and calculated as:

SGR (%/d) = $[(\ln W_f - \ln W_o)/T] \times 100$, where W_f is final weight and W_o is initial weight of the shrimp, and T is the length of the culture period in days. FI was calculated as:

FI = g food eaten / [(W2 + W1)/2].

Data were also assessed as a change in biomass which is the total weight of all animals in a replicate at the second sampling period minus the initial total weight.

Table 1

Ingredients of the nine experimental diets

Ingredient	Percentage of diet (%)
White fish meal	62
Wheat gluten meal	10
Squid meal	8
Mineral mixture	3
Soybean meal	2
Squid oil	2
Soybean oil	1.5
Vitamin premix	0.3
Astaxanthin	0.005, 0.006 or 0.007
Cholesterol	0.3, 0.4 or 0.5
Water and Binder	10.9, 10.8, or 10.7

Photograph and statistical analyses. At the end of the experiment, three randomly selected lobsters from each cage were examined to assess the effect of astaxanthin on spiny lobster coloration. Each lobster was photographed in 4 different body locations, including the exopodite (EXO), endopodite (END), telson (TEL), last abdominal segment medial (LASM) (Tlusty 2005). Photographs were taken with a Sony S730 digital camera (7.2 Megapixels). The flash of the camera was turned off to prevent reflection of light on the bottom surface. The image size was stored in a 1280 x 960 pixel format and saved as JPEG compressed at ¼ the original file size (Tlusty 2005, Tlusty & Hyland 2005). All sample pictures were transferred into Adobe Photoshop software (Photoshop CS2, Adobe, USA). A 1 cm^2 square at each of the four points was used to assess the color changes. Each image was analysed using both RGB and Commission Internationale de l'Eclairage (CIE) L*a*b values (Yam & Papadakis 2004, Chatzifotis et al 2005, Tlusty 2005, Tlusty & Hyland 2005). Following Tlusty & Hyland (2005), only the CIE-L, R, and B values were analyzed. R and B were also combined as a ratio (R/B) since this is the dominant coloration of Astaxanthin during transport and storage within a lobster (Tlusty & Hyland 2005).

All data of weight gain, survival, and color change were analyzed using a two-way analysis of variance (ANOVA), followed by Duncan's multiple range test at the 0.05 level of significance. The results are expressed as mean ± standard deviations (S.D.). The statistical analysis was performed using JMP (version 8.0, SAS, Carey, NC).

Results and Discussion. There was a significant interaction term between astaxanthin and cholesterol levels for the four parameters analyzed (SGR (%d^{-1}) $F_{4,18}$ = 4.19, P<0.05 fig. 1a; FCR $F_{4,18}$ = 3.36, P<0.05, fig. 1b; FI $F_{4,18}$ = 4.61, P<0.001, fig. 1c; and ∆biomass $F_{4,18}$ = 3.23, P<0.05, fig. 1d. This indicates that each variable does not change equally as each dietary parameter increases. From a statistical standpoint, simple effects should be assessed. However, since the astaxanthin and cholesterol need to be fed in combination, a graphical analysis was conducted (figs 1a through 1d) to determine which combination(s) of the two diet components resulted in the best performance.

Overall, lobster fed the 70 mg kg^{-1} (Axt): 0.3 % (Chol), 70:0.4, and 60:0.3 diets were the best performers (largest SGR and ∆biomass, and smallest FCR and FI, Two-way ANOVA, Tukey's HSD, P<0.05). The lobster fed the diets containing 50 mg kg^{-1} axstaxanthin (50:0.3, 50:0.4, and 50:0.5) typically exhibited the poorest performance (fig 1). Lobsters fed the experimental diets also fared better than those fed the trash fish, particularly in terms of FCR where the FCR of lobsters in the comparison cage was 31. While all treatment estimates of survival exceeded the 70 % observed in the trash fish trial, there were no statistically significant treatment differences when levels of astaxanthin and cholesterol were compared (two-way ANOVA, $F_{8,26}$ = 1.10, P>0.4). Survival did exceed 90 % in the 70:0.3, 70:0.4, and 60:0.4 trials and was not significantly different between the nine treatments.

In the present study, a low-level cholesterol supplement demonstrated better growth and survival than those fed higher amounts. This agrees with the study of Williams (2007) who found a dietary level of 0.35 % cholesterol, and supplements up to 0.4 % (Irvin & Williams 2009, Irvin et al 2010) is required for the good growth high survival of juvenile and adult *P. ornatus* lobsters. In other decapods, dietary cholesterol levels for *Homarus americanus* (Milne Edwards, 1837) range between 0.12 % (D'Abramo et al 1984), 0.2 % (Bordner et al 1986), 0.25% (Kean et al 1985), and up to 0.5 % (Castell et al 1975). Previous research findings into *Litopenaeus vannamei* (Boone, 1931) have attained optimal growth when diets contained 0.2–0.4 % (Duerr & Walsh 1996, Gong et al 2000). In another major study, Smith et al (2001) found that the optimum dietary cholesterol requirement for *Penaeus monodon* (Fabricius, 1798) was approximately 0.17 % (dry matter), which is appreciably lower than the current recommendation of 0.25–0.4 %.

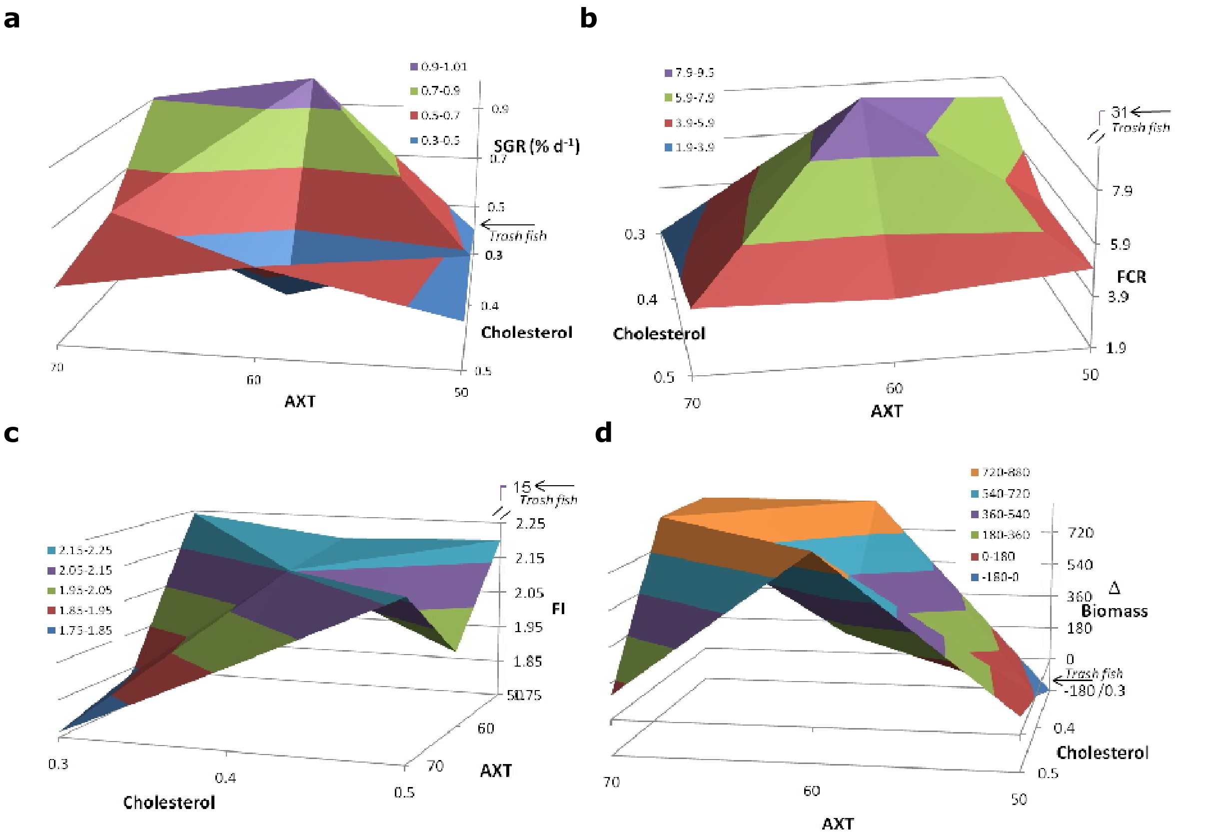
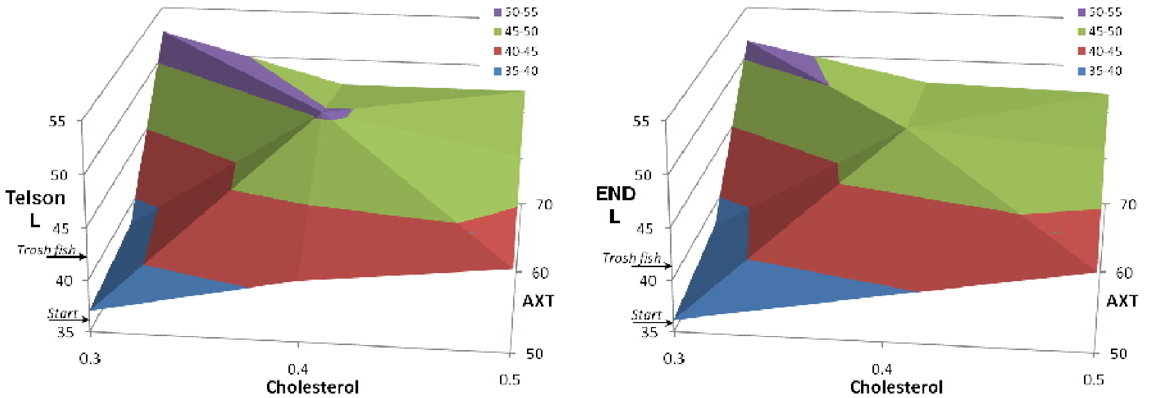


Figure 1. The (a) specific growth rate, (b) feed conversion ratio, (c) daily food intake, and (d) change in total biomass of spiny lobsters fed one of nine diets for 8 weeks. As a comparison, the value of lobsters fed trash fish is identified on the verticle axis. Note that the horizontal axes in graph (c) are switched for ease of data presentation.

One of the most significant and current discussions in improvement of feed and nutrition in aquaculture is feed quality. Traditionally, using "trash fish" (or crabs in this case) has been subscribed to the belief that this practice gives a poor FCR and has caused a drop in water quality due to low stability and resultant wastes from these diets (New 1996). So far, however, there has been little discussion about the artificial diets for spiny lobster in Viet Nam. The present study confirmed that spiny lobster adults can be reared on artificial feed without reliance on a supply of trash fish. Compared to the lobsters fed the trash fish, the FCR, SGR, FI and biomass of lobsters fed the formulated diets improved significantly. In the present study, the food conversion ratio was the best with diet 70:30. The FCR of the trash fish diet is inflated to 31 because the crabs were fed on a wet weight basis. However, assuming 20 % dry matter (Gökoðlu & Yerlikaya 2003), this would reduce the FCR on a dry weight basis to 6.2 which is still greater than any of the experimental diets. Low FCR values can be attributed various factors, including feed quality and feeding, experimental condition, rearing units and size of lobster. Previous workers suggested that the lower the FCR, the better it is because less feed is required per unit weight gain (Pascual 1989).



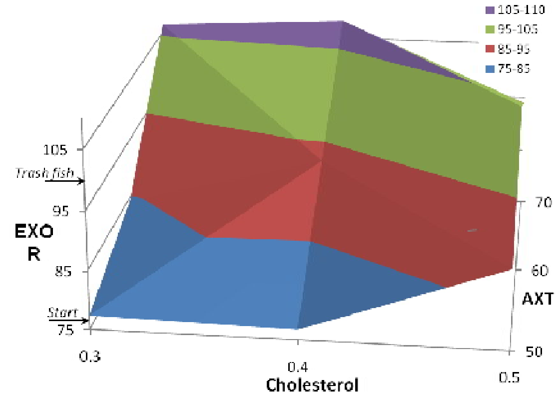


Figure 2. The statistically significant changes in color for spiny lobsters fed one of nine experimental diets. Only the astaxanthin treatment is significant for each of these factors. As a comparison, both the average at the start of the experiment, and the value of lobsters fed trash fish are presented.

Over the 56 day period, the color of the lobsters changed minimally. Of the four measures (L, R, B, R/B) at each of the four locations (telson, exopoidte, endopodite, and last abdominal segment), only three demonstrated significant trends. The astaxanthin treatment was statistically significant for the L value for the telson (two-way ANOVA, $F_{2,18} = 3.98$, $P<0.05$), the L value for the endopodite (two-way ANOVA, $F_{2,18} = 4.12$, $P<0.05$), and the R value for the Exopodite (two-way ANOVA, $F_{2,18} = 4.24$, $P<0.05$). In each of these cases, the lobsters trended toward larger values than at the beginning of the trial, and the trend away from the start value increased with the amount of astaxanthin fed. Lobsters fed the trash fish were intermediate between the start values and the lobsters fed the 70 mg kg^{-1} astaxanthin diet (fig. 2). This result is in contrast to prior results by Barclay et al (2006) that observed that astaxanthin did not affect *P. ornatus* growth or survival.

Conclusions. Formulated aquaculture diets are complex associations of macro and micronutrients designed to promote growth and health of captively reared species. While much work has been divested in determining the optimal inclusion level of nutrients, a majority of this work has been aimed at single nutrient studies. The results of the feeding trial presented within this study demonstrate that, as has been been demonstrated for other species (Ren et al 2009), it is critical to examine nutrient inclusion in feed in reference the other critical nutrients. Here, a benefit of astaxanthin when fed to *P. ornatus* was observed, but the benefit was greatest at low inclusion levels of cholesterol. As diet formulation for lobsters advances during the commercialization of aquaculture production of this species, it will be important to examine micro and macro nutrient inclusion as a suite of factors as opposed to assessing optimization of a single parameter.

This can lead to a better estimation of optimal dietary inclusion levels, as well as revealing significant impacts of nutrients on biological functioning that may not be expressed in a single factor study.

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