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***Research Article***

# EFFECT OF DIETARY ASTAXANTHIN ON SURVIVAL, GROWTH, PIGMENTATION CLOWNFISH, *AMPHIPRION OCELLARIS*, CUVIER

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## ABSTRACT
This study examined influence of dietary Astaxanthin on survival, pigmentation and growth of juvenile false clownfish, *Amphiprionocellaris.* Astaxanthin was added to the squid at four pigment containing 90 mg synthetic astaxanthin (Carophyll Pink 8%)$kg^{-1}$ diet, 180 mg $kg^{-1}$,270 mg $kg^{-1}$ and a control diet (whitount pigmentation). Mean weight of fish were 0.47gr and they were divided into 4 groups. Fish were fed by 7% of their live weight in during of examination for 8 wk. Result is showed that the dietary Astaxanthin could increase coloration of skin and control group had the lightest color but there were no effect on weight length of clown fish significantly (P>0.05).

## INTRODUCTION
The false clownfish, *Amphiprionocellaris,* has been developed for aquarium industry commonly (Hoff 1996; Chapman *et al.*, 1997) it is widely popular in other countries because of its special appearance quality such as beautiful coloration, swimming behavior, its symbiotic relationship with anemone (Yasir and Qin, 2007) and Finding nemo animated. Clownfish have called the "goldfish" of marine aquarium and its value is belonged to skin color (Hoff, 1996). The culture of clownfish has been finished successfully in aquarium (Alva and Gomes, 1989; Maroz and Fishelson, 1997), in fact, the coloration of farm-raised fish is different against of their wild (Booth *et al.*, 2004). The color of hatchery-raised are usually lighter than wild-caught clownfish (Tanaka *et al.*, 1992), but their congener sourced from wild are the more attractive (Allen, 1991). However, everyone can keep farm-raised clownfish in tank for along time and easer than wild-catch. Ornamental fish's pigment is the first parameter dictating their market value. The supplementation Carotenoid can change the clownfish from yellow orange to orange pinkish when the fish were moved from wild to indoor tanks (Tanaka *et al.*, 1992).And the new color could add to the commercial benefit in the aquarium trade (Hoff, 1996; Yasir and Qin, 2009a). Carotenoids play a main role in healthy growth, metabolism, and reproduction, too (Wallat *et al.*, 2005). Anyway, fishes can notsynthesise them like other animals and must be got from dietary sources (Hata, 1972a; Torrissen *et al.*, 1989; Storebakken and No, 1992).The various carotenoid resources have been suggested natural resources such as crustacean and insects (Alavi *et al.*, 2003) and some scientists believed synthetic carotenoids incloding β-carotene, canthaxanthin, zeaxanthin, and astaxanthin can use in daily deitry (Shahidi *et al.*, 1998; Kalinowski *et al.*, 2005). They are easy to obtain and make invariable color (Sales and Janssens, 2003). The main carotenoid is astaxanthin which leads to red-pink coloured aquatic animals and has used in aquacultural, so far.Astaxanthint fish diet has improved skin redness Australian snapper, *Pagruspagrus* skin redness decreased in fish whitoutastaxanthin to the diet of goldfish, *Carassiusauratus*, increased the red pigmentation density of the skin (Xu *et al.*, 2006). Krinsky (1993) reported absorbation of carotenoids enhances fish survival. Yasir and Qin (2009a, 2009b) have explained color of background can change color of skin in the false fish. Therefore, there is no study on the effect of synthetic pigments on the growth of anemone fish. This study was designed to synthetic astaxanthin [Carophyll Pink, Roche] and levels in diets, for their effects on body astaxanthin, survival, and growth of Anemon fish.

## MATERIALS AND METHODS
In this research two-month-old *A.ocellaris* which were produced in aquarium at institute clown fish in Tehran citywere used with average weight of 0.386±0.122 g (Mean±SD), and average total length was

12.7 ± 2.1 mm(Mean±SD). There were twelve 30-L glass aquarias and each aquaria wasstocked with 30 fish. Auariums were recirculated then all factors of water (physically and chemically) were as same as.The background color was choosed blue, intensity of light was 25Lx for14 h-light (Yasir and Qin, 2010).

Salinity and temperature were 27–28%, 26–27 C ordinary.Fish was fed three a day with experimental diet (squid with three doeses from astaxanthin). For 8 weeks and the control group were acclimatized by diet without carotenoid addition. The experimental diet was made in Laboratory at Tehran University of veterinarian department. Three fish were randomly sampled from each aquarium at the end of period. Fish were anesthetized with Clove extract at a concentration of 50 mg/L. That could reduce fish stress and didn't any change color, too.Fish was in this situation for 2 minutes for measuring length, weight and color of skin was compared by color index in figure1.

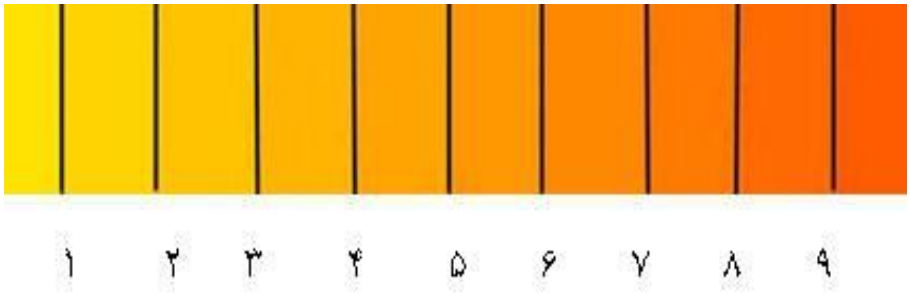


**Figure 1: Clownfish Exercise**

**Mesureing Factors**

1- Body weight increase (BWI), (Tacon, 1990).
BWI=Final weight – Initial weight
2- Percent body weight increase (PBWI), (Bekcan *et al.*, 2006).
PBWI= [(Final weight – Initial weight)/ Final weight] ×100
3- Specific growth rate (SGR), (Hevroy *et al.*, 2005).
SGR=100 × (Ln Final weight-Ln Initial weight / day)
4- Condition factor (CF), (Ai *et al.*, 2006).
CF=[Weight(g)/$\left(Length(cm)\right).^{3}$ ] × 100
5-Day growth rate (DGR), (De Silva and Anderson, 1995).
DGR=[( Final weight – Initial weight)/day ]×100
6-Feed conversion ratio (FCR), (Ai *et al.*, 2006).
FCR=feed intake(g)/weight gain(g)
7-Survival rate (Ai *et al.*, 2006).
Survival rate =100× (Initial fish number-dead fish number)/(Initial fish number)

***Statistical Analysis***

All analysis was performed using SPSS 19.0 for Windows statically. One-way ANOVA in significant level of difference at $P$ <0.05 was used and average mean was compared by Duncan's multiple range test.

## RESULTS AND DISCUSSION

***Results***

There was showed that the carotenoid affected the color skin of fish significantly. At the beginning, all fish had colouration in the ranges 2 to 3 (figure1).

**Table 1: Appearance comparing color of skin according to index at figure 1**

| | |
|---|---|
| Control | 2-3 |
| 90 ppm | 4-5 |
| 180 ppm | 8-7 |
| 270 ppm | 9 |

After 4 weeks, colour changes appeared in all the groups exactly. However after 8 weeks, the most colouration was found in the fourth group which fed by 270ppm dose astaxanthin and that color was darker than part 9 in order to Table-1. There was no observed survival and growth no mortality occurred throughout the experiment.

**Table2: Measured factors in during experimentation**

| **Factors** | **Control group** | **Treatment1 (90AX)** | **Treatment2 (180AX)** | **Treatment3 (270AX)** |
|---|---|---|---|---|
| BWI(gr) | 0.8711 ± 0.066 [a] | 0.9385 ± 0.034 [a] | 1.0343 ± 0.032 [a] | 1.0057 ± 0.076 [a] |
| PBWR | 71.86 ± 1.5 [a] | 73.73 ± 0.633 [a] | 75.56 ± 0.588 [a] | 74.92 ± 1.442 [a] |
| SGR | 0.847 ± 0.036 [a] | 0.891 ± 0.015 [a] | 0.939 ± 0.015 [a] | 0.924 ± 0.038 [a] |
| DGR | 0.580 ± 0.044 [a] | 0.625 ± 0.023 [a] | 0.689 ± 0.021 [a] | 0.670 ± 0.051 [a] |
| CF | 2.029 ± 0.083 [a] | 1.841 ± 0.051[a] | 2.201 ± 0.028 [a] | 2.167 ± 0.039 [a] |

In order to table-2 there are no significant different between tretments group and control but the most body weight increase is showed in treatment 3 ($p<0.05$).

***Discussion***

Our results agree with the study of Bell *et al.,* (2000) that dietary ataxanthin did not affect significant growth and survival of Atlantic salmon (Salmosalar). Alsoastaxanthin had no differences in growth of rainbow trout Amar *et al.,* (2001). According to Gomes *et al.,* 2002 growth of gilthead seabream were no change when fed with synthetic astaxanthin or *Haematococcuspluvialis*and a similar result was obtained in this study of Gouveia *et al.,* (2003) growth of koi carp and gold fish were not different when they used C. vulgaris, H. pluvialis, and cyanobacteriumArthrospira maxima (Spirulina) and our result admire Amar *et al.,* (2001) fed rainbow trout with Dunaliellasalina which contains BC, and Phaffiarhodozyma which contains astaxanthin and found no difference in growth rate between the treatment groups and the control group. Dietry with astaxanthin and β-caroten could no change growth of *Hyphessobryconcallistus* (Wang *et al.,* 2006).

However, in the study by Kim *et al.,* (1999), the Korean rose bitterling (Rhodeusuyekii) fed AX supplemented diet showed higher growth rates than those fed lutein or BC and the control. In general, there are no positive effects observed on growth of penaeids fed with carotenoid supplemented diets (Yamada *et al.,* 1990; Chien and Jeng, 1992; Negre-Sadargues *et al.,* 1993; Liao *et al.,* 1993, Mensaveta *et al.,* 1993; Pan *et al.,* 2001). In this study showed the highest growth belonged to two groups which fed with 180 and 270 ppm, however they had no significant differences.

Fish were alive in during period of experimantion and we showed no death between them. In this case survival of fish was independ on supplemented carotenoid. But Christiansen *et al.,* (1995) reported there

were significant differences in survival of atlanticsalmo which fed with dietry of astaxanthin opposite of control group and in other research by Christiansen and Torrissen (1997) on Atlantic salmon showed the same result with old research. Carotenoid decreased survival of adult shrimp significantly (Yamada *et al.,* 1990). Dietry of astaxanthin and β-caroten effected on survival of Japeness shrimp in one month and amount of survival was higher in group which fed with astazanthin ratio to group which used supplemented β-caroten (Chein and Jeng, 1992). But Pagrus *et al.,* (2007) reported astaxanthin had no affectes on survival of *Tejera*fish that agree our result.

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