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Effect of Different Levels of Astaxanthin on Shell Color and Growth Indices of Freshwater Crayfish (*Astacus leptodactylus* Eschcholtz, 1823)

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Abstract: A feeding trial was conducted for 60 days duration using Crayfish (*Astacus leptodactylus*) to evaluate the diet contain 0(control), 50, 75 and 100 $mgkg^{-1}$ of synthetic astaxanthin. 120 crayfish of 20±2.7 g size were randomly distributed between 12 fiberglass tanks of 500L capacity. All crayfishes fed astaxanthin as carotenoid sources supplemented diets displayed from brown to greenish shell after 60 days of feeding in contrast to black appearance displayed by crayfish fed with basal diet (0). Growth performance in the term of weight gain (WG), food conversion ratio (FCR) and survival rate (SR) was best in diet contain l00 $mgkg^{-1}$ of astaxanthin and showed significant differences with other diets ($P<0.05$). There was no significant differences in WG and FCR in control and T1 and T2 treatments (0, 50 $mgkg^{-1}$ and 75 $mgkg^{-1}$, respectively); however survival rate (SR) in T_1 and T_2 improved significantly as compared with control diet ($P<0.05$). Crayfish carcass composition was improved in diet contain 100 $mgkg^{-1}$ as astaxanthin and showed significant differences with other diet ($P<0.05$); however, there was no significant differences in control, T_1 and T_2 in body composition. It can be concluded that the most appropriate dietary doses of synthetic astaxanthin for pigmentation and best growth performance of crayfish is 100$mgkg^{-1}$.

Key words: Freshwaters crayfish · Astacus leptodactylus · Astaxanthin · Growth performanc

INTRODUCTION

Nutrition has an important impact on several parameters directly influencing the quality of fishes and shell fishes such as colour and appearance; meanwhile most consumers tend to prefer brighter colour of fish or shell - fishes (crayfish, prawn and etc) [1, 2].

Although the biosynthesis of carotenoids can be carried out by plants, algae and bacteria, this kind of natural pigment is also found in animals like fish and shell-fishes which live in natural environment [3]. Since the coloration and even quality of fishes and shell-fishes degrade in confined or cultured area due to insufficient natural food; therefore addition of carotenoid sources in the form of synthetic astaxanthin or maybe in the form of natural one which extracted from prawn, crayfish and crab shell is essential to enhance coloration and flesh quality [4, 5].

Carotenoids, especially astaxanthin are responsible for the typical colour of salmonid muscle as well as for prawn; in addition they act as anti-oxidant precursor of vitamin A [6-8]. Carotenoids are responsible for the various colors of crustaceans, they have attracted many researchers because of their commercially desirable properties, such as their natural origin, null toxicity and high versatility providing both lipid and hydro-soluble colorants and pro-vitamin A activity [9]. Many studies on comparative pigmentation efficieny between astaxanthin and canthaxanthin in rainbow trout which has always been assumed that rainbow trout was a good model for all salmonid or other fishes [10, 11]. However, study of the carotenoid sources effect on shell fishes like prawn, comparatively is scarce and available knowledge is mostly regarding to the extraction of carotene from prawn and crayfishes wastes [3]. Meanwhile, the main carotenoid found in shrimp waste has important

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application in the functional food, feed for crustaceans and salmonidae, cosmetic and food industries and has a possible role in human health [12]. Reports on the specific dietary carotenoid sources in the form of astaxanthin requirement of fresh water crayfish are, however, scanty.

The present study was designed to assess the relative pigmentation efficiency of astaxanthin doses in diets for fresh water crayfish (*Astacus leptodactylus*), raised in confined or control area; in addition, from the point of commercial value, the attractive color of crayfish fetches higher price in market, especially for export purposes.

MATERIALS AND METHODS

Supply and Maintenance of Crayfish: Crayfish (*Astacus leptodactylus*) were provided by Sefidruod Fisheries Research Station and transferred to wet-lab of Faculty of Natural Resources of Guilan University. Initially all crayfishes (during an acclimatization period) were stocked in 5 ton tanks without feeding for 48 hrs. One-hundred and twenty crayfish of 20±2.6 g size were randomly distributed between 15 tanks of 500 l capacity (100 cm dia, 50cm height) with 10 per tank (meant, n-10). Crayfish were feed at 5% of their body weight per day in 4 times (8, 12, 16 and 20 hr). Animals were weighed once every week and daily ratio adjusted accordingly. The growth trial was conducted in July and August under natural photo period (light/dark cycle of 12h: 12h). Water parameters like temperature, pH and Do were 25.4±2.7°C, 7.5±0.41 and 6.6±0.5 mg/l during months of trial.

Diet Design: The formulation proximate compositions of ingredients used in this experiment are shown in Table 1 and 2. All common natural ingredients were procured from local market in one batch, dried, ground, sieved and stored in air tight containers. First, dry ingredients (natural and purified ingredient) mixed for 15 min before addition of oil mixture and premixes; after mixing the oil and premixes for 10 min; then these materials were added to final mixture and again mixes for 15 min.

Table 1: Proximal composition (as fed basis) of the ingredients used for feed [34] Values are mean±S.E.

Ingredients	CP (%)	CF (%)	EE (%)	NFE (%)	Moisture (%)
Egg-Albumin	92±0.3	0.00	0.00	1.20±0.1	2.33
Gelatin	96.5±0.25	1.65±0.02	0.00	2.0±0.36	0.28
Dextrin	0.98±0.003	1.25±0.035	0.07±0.0	94.47±0.24	0.47
Fish meal	61.8±0.7	0.88±0.01	6.98±0.3	5.79±0.8	4.85
Shrimp meal	41.9±0.9	4.92±0.8	4.9±0.7	5.35±0.61	4.16
Squid meal	68.7±0.8	0.8±0.001	4.22±0.42	26.1±0.14	2.76
Diets	35±0.71	6.8±0.32	4.1±0.44	9.2±0.31	10.2±0.27

CP = Crude Protein (NX 6.25)

NFE = Nitrogen Free Extract

Table 2: Percentatage composition of ingredients of experimental diets and their proximate analysis (as fed basis) (Nutrition lab of Guilan University).

Ingredients (%)	Control	T_1	T_2	T_3
Egg - albumin	12.00	12.00	12.00	12.00
Gelatin	9.00	9.00	9.00	9.00
Dextrin	31.00	31.00	31.00	31.00
Fish meal	10.00	10.00	10.00	10.00
Shrimp meal	10.00	10.00	10.00	10.00
Squid meal	10.00	10.00	10.00	10.00
Sun flower oil	6.00	6.00	6.00	6.00
Fish-oil	3.00	3.00	3.00	3.00
Vit mix	2.00	2.00	2.00	2.00
Mineral mix	3.00	3.00	3.00	3.00
Binder	2.00	2.00	2.00	2.00
Anti-fungi	0.25	0.25	0.25	0.25
Anti-oxidant	0.02	0.02	0.02	0.02
Lecithin	1.00	1.00	1.00	1.00
Vitamin C*	0.10	0.10	0.10	0.10
MCP	0.40	0.40	0.40	0.40
Astaxanthin ($mgkg^{-1}$)	0.00	50.00	75.00	100.00

*: Noverian [34] expect inclusing of astaxautin proximate analysis (SDI)

Three-hundred and fifty ml distilled water added to mixed ingredients to obtain mash, then mash was slow cooked (steam cook with out pressure) for 15-20 minute to obtain stiff dough. This dough extruded through a kitchen noodle maker with 2mm dia to obtain strand (2×1.5 mm). The strands oven dried for 24 hr at 60°C to reduce moisture to 10%. The pellets for all diets (0, 50, 75 and 100 $mgkg^{-1}$ of astaxanthin) were produced in the same way. The ingredients were first turned in to a homogenous doughy consistency by adding water and converted in to pellet form by being pressed through grinding machine with 2 mm die. The experiment was ended at day of 60 and final weights were recorded and crayfish carcass was analyzed for crude protein, lipid, ash and moisture. All ingredients, food and crayfish samples (carcass) were analyzed according to A.O.A.C [13] at nutritional lab of faculty of natural resources.

Pigment Analysis: Dissection of fish and extraction of carotenoids were carried out as described by Torrissen and Naevdal [14]. Five crayfish taken from each diet treatment were used for each carotenoid analysis and these analyses were carried out duplicate. Shell of crayfish tail removed for pulp (flesh) extraction, the remain muscle samples were minced with a meat mincer and samples of approximately 2-3g were taken for analysis and then transferred to 10ml pre-weighted glass tubes. First, 10ml of dry acetone and then about 2g of anhydrous sodium sulphate were added to the samples. The solution were centrifuged at 5000g for five minute and stored at 4^0 C in refrigerator. After 3 days of extraction in sealed glass tubes, the absorption of the extracts was measured at 476nm in a spectrophotometer. A similar method was used for total astaxanthin analysis.

The Following Parameters Were Estimated:

- Initial (WI) and final (WF) body weighed to nearest 0.1gr were recorded.
- Weight gain (WG) g = final weight (g) – Initial weight (g).
- Food conversion ratio (FCR) = dry food offered (g)/ wet weight gain (g).
- Survival rate (SR) = Final number of crayfishes / Initial number of crayfishes.

Statistical Analysis: Raw data were analyzed by one- way ANOVA. Duncan multiple range test has employed to find-out which treatment different significantly from each other with respect to growth indices and muscle composition at $p<0.05$. Statistical analysis was conducted using SPSS (Version 13, Inc., Chicago IL, USA).

RESULTS

Total carotenoid contents in the fish muscle were presented in Table 3. Diet groups were significantly highest extraction of carotenoid fed with 100 $mgkg^{-1}$ of astaxanthin and showed significant with other diet ($p<0.05$). Carotenoid contents in crayfish muscle fed

Table 3: Total carotenoid content ($mgkg^{-1}$) and Growth indices of freshwater crayfish (*Astacus leptodactylus*) fed experimental diets with astaxanthin for 60 days. Values are mean±S.E.

	Total Carotenoid content ($mgkg^{-1}$)						
Diets	Day 0 (Initial)	Day 60 (Final)	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR (g)	SR (%)
Control	0.898	1.338±0.027^c	20.2±0.32^b	27.4±0.64^b	7.2±0.43^b	1.8±0.14^b	75±3c
50mg kg^{-1} astaxanthin (T_1)	0.898	4.234±0.07^b	19.9±0.2,9^b	27.9±0.46^b	8±0.65^b	1.79±0.17^b	86±2^b
75mg kg^{-1} astaxanthin (T_2)	0.898	4.635±0.063^b	19.8±0.61^b	27.6±0.41^b	7.8±0.61^b	1.74±0.18^b	85±3^b
100mg kg^{-1} astaxanthin (T_3)	0.898	6.543±0.029^a	20.6±0.76^a	32.1±0.52^a	11.5±0.72^a	1.45±0.17^a	92±2^a

Values having the different superscript in the same column are significantly different ($P<0.05$)

Table 4: Proximat muscle composition (on-wet basis) of fresh water crayfish (*Astacus leptodoctylus*) fed experimental diets whit Astaxanthin for 60 days. Values are mean±S.E.

		Final values			
Muscle composition (%)	Initial value	Control	T_1(50$mgkg^1$)	T_2(75$mgkg^{-1}$)	T_3(100$mgkg^{-1}$)
Moisture	68±3	66±2^a	65.6±3^a	65.32^a	60±2^b
Protein	19.82±0.78	20.40±0.71^a	20.52±0.61^a	20.68±0.46^a	23±0.51^b
Lipid	6.31±0.78	6.80±0.46^a	6.82±0.33^a	6.84±0.35^a	8.78±0.41^b
Ash	8.78±0.91	8.56±0.35^a	8.51±0.43^a	8.45±0.34^a	6.12±0.39^b

Values having the different superscript in the same row are significantly different ($P<0.05$)

each diet supplemented with a pigment source were significantly higher than those of control basal diet ($p<0.05$). At onset of the experiment, the carotenoid level in the muscle was 0.898mgkg^{-1}; this value had risen to 6.543mgkg^{-1} by the end of the experiment. Growth indices in the term of WG, FCR and SR were improved when dietary astaxanthin level increased to100 mgkg^{-1} in treatment 3 (T_3) and showed significant with other treatment (Table 3). No significant differences in growthperformance were found between control, T_1 and T_2; however, survival rate in T_1 and T_2 was improved and showed significant with control diet ($p> 0.05$).

Muscle composition in crayfish is presented in Table 4. There were no significant differences in muscle content in control diet, T_1 and T_2; however, with increasing astaxanthin level to 100 mgkg^{-1} in T_3, protein and lipid increased from initial value, while moisture and ash decreased from initial and showed significant with others ($p<0.05$).

DISCUSSION

The available information on astaxanthin use in fresh water crayfish (*Astacus lepotodactylus*) is limited. The available data which has been worked out by scientists on macro-nutrients of fresh water crayfish used as a base line for food formulation as well as water quality maintance [15, 16]. Color is an important characteristic and selection criterion for food choice by consumers; recent studies have high lighted may change among certain population and over time [17]. Different pathways have been proposed concerning the formation and evolution of carotenoids in Crustacea; most imply b-carotene and/or the xanthophylls thus deriving, as astaxanthin precursors [18-22]. Lutein is also largely reported in Crustacea; most fresh water species are found to contain rather important amounts of this carotenoid [23-25]. Most freshwater species transform ingested carotenoids into astaxanthin, accumulating this carotenoid in their tissues before its transfer to the previtellogenic oocytes [26]. The mode of development of the crayfish, lacking of larval period, represents a marked difference toward marine astacidea, like lobsters, which exhibit several larval instars, preceding the appearance of the juvenile individual [26]. This circumstance promotes the idea that the typical carotenoid pattern of the adult crayfish, appears earlier as a result of embryonic metabolic activity occurring before the eclosion, whereas in lobsters and most marine crustaceans, the adult pigmented pattern is conditioned by the feeding activity of hatched larvae and the progressive development of the metabolic machinery [27].

Barbosa *et al.* [28] reported that rainbow trout with initial body weight of 150g fed experimental diets supplemented with 100 mgkg^{-1} of astaxanthin showed best. Performance, which almost coincide with our result in fresh water crayfish which performed best growth, survival rate and FCR at 100 mgkg^{-1} of astaxanthin. Meanwhile the European commission heath consumers (ECHC) (2002), recommended maximum 100 mgkg^{-1} of astaxanthin for trout, but dose recommended for crustaceans not available yet, need further work.

Choubert and Storebakken [29] proved that the addition of carotenoid sources (astaxanthin and canthaxathin) in fresh water trout improved pigmentation and give attractive color; in the same result we also found that the inclusion of astaxanthin in the diet of crayfish improved color from black (0 astaxanthin) to brown and greenish coloration. Schiedt [30] reported that there is no any harmful effect of synthetic carotenoid sources in crustacean.

Growth performance in term of WG and FCR showed no significant differences between control diet (0), T_1 and T_2 (50 and 75mg kg^{-1}). This maybe due to insufficient requirement of astaxanthin in the diet of fresh water crayfish, but, there is no any report about dose using of crayfish for comparison. However, Baker *et al.* [10] performance in rain bow trout was 100 mgkg^{-1} astaxanthin in its diet. Although there was no significant differences in WG and FCR in control, T_1 and T_2, but survival rate in T_1 and T_2 improved significantly as compared with control diet may be due to effect of astaxanthin as antioxidants and precursor of vitamin A [7, 14].

Schiedt [31] proved that the optimum duration for absorption, retention and metabolic transformation of carotenoids in salmonids and crustacean is 60 days, which coincide with our result of 60 days trial duration for perfect effect.

Moisture and ash content decreased, where as, protein and lipid increased from initial value.

In T_3 (100mg kg^{-1}) of astaxanthin uses and showed significant difference with other diets. Matsuno *et al.* [32] reported that the inclusion of carotenoid sources in optimum level (>80mgkg^{-1}) has improved body composition of salmon.

Sachindra *et al.* [33] also proved that addition of carotenoid sources in the diet of shrimp improved body component, the above results coincide with our findings.

From the result of the present study it can be concluded that the optimum level of astaxanthin in the diet of fresh water crayfish for pigmentation (attractive color) and best growth performance is 100mg kg^{-1}. This survey also formed a base line in using astaxanthin for other commercial crustacean, which need further study.

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