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Research Article

Effects of dietary astaxanthin and β-carotene on gonadosomatic and hepatosomatic indices, gonad and liver composition in goldfish *Carassius auratus* (Linnaeus, 1758) broodstocks

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ABSTRACT. This study investigated the effects of two source carotenoids on the hepatosomatic index (HSI) and the gonadosomatic index (GSI) of the goldfish (*Carassius auratus*). For this experiment, six diets containing concentrations of 50, 100, and 150 mg carotenoid kg^{-1} of synthetic astaxanthin and β-carotene were added to a basic carp diet, while one group of fish was fed a control diet (no added carotenoids). Fiberglass tanks were used for the study, which was performed from January to April of 2013. During the breeding period, the hepatosomatic (HSI) and gonadosomatic (GSI) indices were exa- mined monthly, along with the total carotenoid levels in the ovaries and livers of female goldfish broodstock. A significant positive correlation was observed between total carotenoids in both the liver and ovary tissues and GSI after 90 and 120 days of the experiment. The HSI was not affected by the carotenoid-enriched diets. However, the carotenoid-enriched diets resulted in greater accumulation of carotenoids in the gonads. β-carotene-enriched diets (B150) led to greater accumulation of carotenoids in the liver after 90 days. Lastly, we found that diets containing higher levels of astaxanthin and β-carotene improve the GSI in goldfish broodstock.

Keywords: *Carassius auratus*, goldfish, carotenoids, sexual maturity, astaxanthin, β-carotene.

Efecto de la astaxantina y β-caroteno en la dieta, sobre los índices gonadosomático y hepatosomático, composición de la gónada e hígado en reproductores de carpa dorada *Carassius auratus* (Linnaeus, 1758)

RESUMEN. Este estudio investiga el efecto de dos fuentes de carotenoides sobre el índice hepatosomático (HSI) y el índice gonadosomático (GSI) de la carpa dorada (*Carassius auratus*). En este experimento, se adicionaron seis dietas conteniendo concentraciones de 50, 100 y 150 mg carotenoide kg^{-1} de astaxantina sintética y β-caroteno a una dieta de carpa, mientras a otro grupo de peces se le suministró una dieta control (sin carotenoides). Se utilizaron estanques de fibra de vidrio de enero a abril 2013. Durante el periodo experimental, el HSI y el GSI se examinaron mensualmente junto con la concentración de carotenoides totales en ovario e hígado de hembras. Una correlación positiva significativa se encontró entre el total de carotenoides aportados en la dieta y el hígado, ovario y el GSI después de 90 y 120 días de experimento. El HSI no fue afectado por las dietas enriquecidas con carotenoides. Sin embargo, las dietas con carotenoides mostraron un mayor acumulación de carotenoides en las gónadas. La dieta con β-carotenos (B150) presentó una mayor acumulación de carotenoides

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en el hígado después de 90 días. Finalmente, se encontró que las dietas que contenían el nivel más alto de astaxantina y β-caroteno mejoraron el GSI en los reproductores de carpa dorada.

Palabras clave: *Carassius auratus*, carpa dorada, carotenoides, madurez sexual, astaxantina, β-caroteno.

INTRODUCTION

The freshwater goldfish *Carassius auratus* is the most popular aquarium fish species and has a high market value (Lee & Newman, 1997). According to Yanar *et al*. (2008), ornamental fish production contributes to economic development in developing countries, especially in the tropics. Based on 2011 statistics, however, Europe is currently the global leader in goldfish aquaculture production at 2,469 ton, followed by Asia, which produced 126 ton (FAO, 2013).

Modern scientific techniques and methods have improved the propagation of aquatic animals (Izquierdo & Fernandez-Palacios, 1997). In addition to providing suitable environmental conditions, broodstock nutrition is one of the main factors affecting the reproductive success of aquatic organisms. It not only affects the maturation and production of high quality gametes, it also plays a role in survival rates and quality of larvae (Wooster & Bowser, 2000).

Broodstock nutrition affects gonadal development and fecundity in fish (Watanabe & Vassallo-Agius, 2003). To improve the quality of fish reproduction, different dietary supplements, such as vitamins, lipids and carotenoids are used (Chainapong & Traichaiyaporn, 2013).

Carotenoids are pigments found in plants and animals, but only plants, including algae, some bacteria and yeasts are able to produce them. Nonetheless, structural variants of carotenoids can still be found in animals. Perciformes and Salmonidae fish synthesize zeaxanthin from astaxanthin; however, this is reversed in Cyprinidae fish, with astaxanthin being synthesized from zeaxanthin (Maoka, 2011). Research has shown that carp fed high-carotenoid diets retained serum astaxanthin and red color better than did other groups and had a higher phagocyte concentration (Yuangsoi *et al*., 2010, 2011). Additionally, carotenoids are believed to be essential for reproduction in aquatic animals. Studies have shown that carotenoids can directly affect the rate of glycogen storage and increase glycogen concentration in the liver of fish (Barber *et al*., 2000). Through accumulation in the liver and subsequent transport to the ovaries and the oocyte, carotenoids induce changes that improve broodstock maturation (Kerfeld *et al*., 2003). Astaxanthin supplementation of the broodstock diet of fish improves reproduction performance, floatation and hatching of eggs, and larvae health (Sawanboonchun *et al*., 2008). It has been shown that astaxanthin and β-carotene improves the reproductive performance and egg quality of female goldfish (Tizkar *et al*., 2013). Additionally, researchers suggest that carotenoids have respiratory functions in fish eggs (Craik, 1985). However, it is important that the effects of carotenoids, in the gonads and the liver, are studied over time to understand the incorporation of astaxanthin.

Gonadosomatic and hepatosomatic indices were used as indicators of exposure effects, from contaminants carried in sewage effluents, on carp (Diniz *et al*., 2005), and to determine the toxicity of micronutrient (Librel® highly soluble chelates for plant nutrition) on tilapia (Sadekarpawar & Parikh, 2013). Histology of the ovaries of female fish reveals that they are more sensitive than males in regard to the GSI index. The goal of this study was to evaluate the effects of a carotenoid-supplemented diet on the GSI, HSI and on gonad and liver composition in goldfish (*C. auratus*) broodstocks.

MATERIALS AND METHODS

Diet treatments

The composition of the diets followed those described in Tizkar *et al*. (2013). Three concentrations (50, 100, and 150 mg kg^{-1}) of two carotenoids sources - astaxanthin (A50, A100 and A150) and β-carotene (B50, B100 and B150)- were used for the carotenoid-enriched diets. The control diet (Control) did not contain added carotenoids. In total, seven treatments, in triplicate, were used in this experiment.

Diets enriched with synthetic astaxanthin (Carophyll® pink 10%, DSM-Bright Science, Brighter Living™, Netherlands) and β-carotene (β-carotene 10% CWS, Direct Food Ingredients, UK) were weighed, dissolved in water at 35°C, and added to the diet according to each tested level (Page & Davies, 2003). All diets were prepared with fish oil and water and processed in a California Pelleting Machine (CPM), producing pellets at a size of 2x3 mm in diameter. Pellets were then dried in a drying machine (Hobart Manufacturing Company Ltd., London, UK) for 24 h at 50°C. All diets were placed individually in black plastic bags and stored at 18°C. Diets were prepared once every fifteen days. The amount of total protein (33.6-34.5%), fat (7.67-8.25%), ash (8.98-9.31%) and moisture (5.69-5.77%), were measured according to procedures described by AOAC (2000).

Broodstock

One-year-old broodstock goldfish were used for the experiment. The average weight and length of the fish were 47.21 ± 2.19 g and 14.71 ± 0.31 cm, respectively. Fish were reared at the Rasht Bony Fish Hatchery complex in Rasht, Iran and fed a commercial diet before being fed the experimental diet.

Experimental trials

Trials were conducted in 21 fiberglass tanks (3.77 m^3; 2 m diameter and 1.2 m height) and lasted for 120 days (December 2012 to April 2013). The water temperature (YSI 55 Yellow Springs, Ohio, USA), dissolved oxygen content (YSI 55), pH (Oakton pH Tested) were measured daily. Nitrite and ammonia concentrations (YSI 9000 photometer, Series analysis kit, Yellow Springs, Ohio, USA) were measured weekly for the duration of the experiment.

Prior to the experiment, the broodstocks were transferred to storage tanks and disinfected with 5% saline water. To accustom the fish to the control diet, they were fed the control diet two times per day (10:00 and 15:00) for a period of one week. After this period, the female broodstocks were removed from the storage tanks and distributed randomly in the experimental tanks, with 50 individuals in each tank. Over the course of the experiment period, 10% of the water in each tank was renewed daily and the animals were fed twice daily with 2% of the biomass.

Somatic and total condition index

The broodstock from each treatment were sampled on a monthly basis. After sampling, the body, liver and ovary of each female fish were weighed for inclusion in the hepatosomatic and gonadosomatic indices. The HSI and GSI were calculated using the formula described by Barber & Blake (2006):

HSI = [liver weight (g) / fish weight (g)] x 100

GSI = [gonad weight (g) / fish weight (g)] x 100

Sampling and analytical methods

Liver and ovary tissue samples (1.5 g; 6 ind) were collected, frozen (-80ºC for 24 h) and stored in liquid nitrogen before the start of the experiment (T0) and at each sampling time (T30, T60, T90 and T120). After the sampling period, the frozen samples were dried at -55ºC (Christ Freeze Dryer-Alpha-1-2 LDplus model) and stored at -18°C in an amber flask filled with N_2 for later carotenoid extraction (Sühnel *et al*., 2009).

Total carotenoid determination

The total carotenoids in the ovary and liver were analyzed. The total carotenoids were extracted and quantified at the Biochemistry Laboratory of the Sharif University of Technology in Tehran, Iran. For this analysis, 50 mg (dry tissue weight) samples were taken from each tissue type and added to 5 mL of an extracting solution containing pure acetone (Ac = O, analytical grade) and n-hexane (Hex, analytical grade) at a ratio (v/v) of 1:3, respectively. This mixture was then agitated in a magnetic agitator (10 min) at ambient temperatures (22°C) and under dark conditions (Sühnel *et al*., 2009).

Carotenoid concentration in the extracted solution was determined according to procedures described by Sühnel *et al*. (2009), a UV-visible spectrophotometer (Hitachi, U-1800) with the spectral window set between 380 and 750 nm was used, in triplicate. Carotenoid concentration was derived using the Lambert-Beer law and for calculation purposes the following equation was applied to the absorbance values:

Total carotenoid (µg mg^{-1}) = [absorbance/ε x molecular mass x 1000 x sample volume (mL)]/ sample dry weight (kg)

Based on a specific optical extinction coefficient ε^{1}_{1cm} of $124{,}000_{(astaxanthin)}$ at 460 nm and a molecular mass of 596.84 (astaxanthin).

Statistical analysis

Data in the present research are reported based on average replication (mean ± standard error). The normality of the data was evaluated using the Kolmogorov-Smirnov test, while homogeneity was assessed using the Levene test. ANOVA was used to compare the HSI and GSI, followed by Tukey's *post-hoc* test (Zar, 2010), using Statistica version 10.0 (Statsoft, USA) software.

RESULTS

Water quality

No significant differences ($P < 0.05$) were observed for pH (7.60 ± 0.12), dissolved oxygen (7.10 ± 1.20 mg L^{-1}), nitrite (<0.01 ± 0.01 mL L^{-1}) and ammonia (0.01 ± 0.01 mL L^{-1}) during the period (120 days) of female maturation. However, a significant difference was detected for temperature, which started at 12.16 ± 0.22°C, dropped to 6.57 ± 0.38°C at T60, and then rose to 20.85 ± 0.38°C at T120.

Hepatosomatic and gonadosomatic indices

The relationship between HSI and GSI is presented in Figure 1. There was a significant negative relationship between both indices ($R^2 = 0.88$; $P < 0.05$). Periodic variations in the GSI and HSI of females due to additions

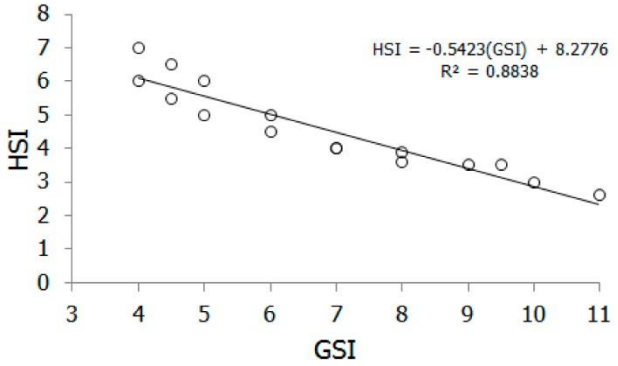

Figure 1. Relationship between the gonadosomatic index (GSI) and the hepatosomatic index (HSI) in female of *C. auratus*.

of astaxanthin and β-carotene are shown in Figure 2. The HSI values for females at 30 and 120 days were 7.02 ± 0.34 and 2.48 ± 0.04, respectively, compared to the control (6.0 ± 0.22 to 2.30 ± 0.03). The A50 treatment was the lowest value at T120 (3.14 ± 0.14). In general, there were no significant differences ($P > 0.05$) between the treatments and the control. The HSI values for the β-carotene-enhanced diet at 30 days and 120 days were 6.7 ± 0.23 and 2.65 ± 0.06, respectively, compared to the control (2.52 ± 0.03), with the B150 treatment having the lowest value at T120 (2.19 ± 0.12).

The GSI showed a trend opposite to that of the HSI, with the A150 treatment having the highest value at 120 days (12.71 ± 0.84) and the A50 treatment having the lowest value at 30 days (3.02 ± 0.41). The treatments with the highest GSI were A150 (12.71 ± 0.84) and B150 (12.07 ± 0.60), both of which were significantly different ($P < 0.05$) from the control (8.3 ± 0.52).

Total carotenoids in the liver and the gonads

Analysis of the data collected at each sampling time shows that the total carotenoid content in the liver was significantly different within T90 ($P < 0.05$) and T120 ($P < 0.05$) (Fig. 3). At T120, the A150 treatment was significantly different (6.80 ± 0.22 μg mg^{-1}) from all of the other treatments and the control. Otherwise, the total carotenoid content of the gonads was significantly different at all sampling times. In general, the carotenoid concentrations in the gonads were higher in the first 30 days of the experiment and decreased until the final sampling time (120 days).

Analysis of each treatment (Fig. 3) shows that the total carotenoid in the liver was significantly different ($P < 0.05$) from the control. The highest levels of total carotenoids at T30 were observed for the A50 treatment (56.68 ± 4.27 μg mg^{-1}). For the A100, A150, B100 and B150 treatments, the highest levels of total carotenoid were recorded at day 30 (T30) (66.98 ± 4.42 μg mg^{-1}, 85.58 ± 5.47 μg mg^{-1}, 55.67 ± 4.28 μg mg^{-1} and 68.84 ± 3.64 μg mg^{-1}, respectively).

Correlation between the HSI, GSI and total carotenoids

The correlation between the HSI, GSI and total carotenoid concentrations in the liver and in the gonads

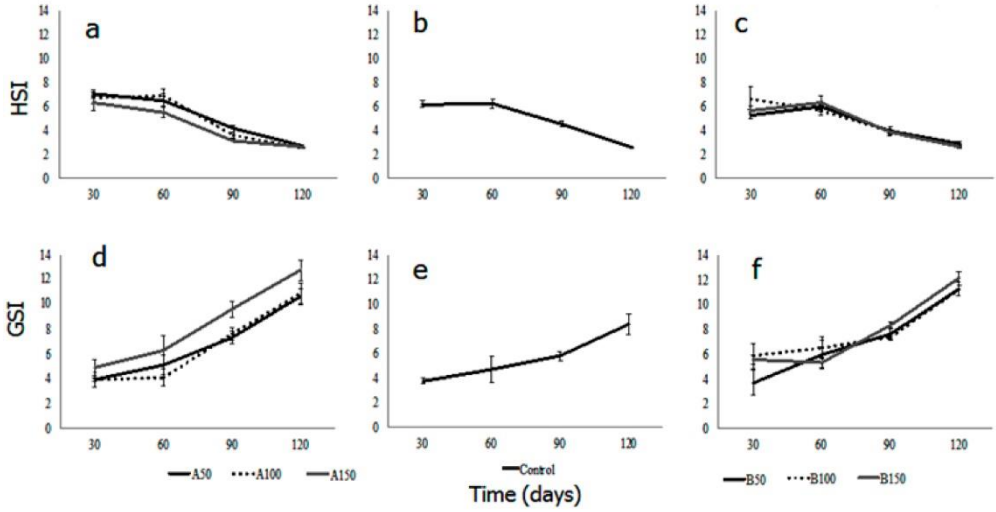

Figure 2. Temporal variation of the hepatosomatic index (HSI) and the gonadosomatic index (GSI) of *C. auratus* for the treatments with astaxanthin, β-carotene and the control. a) HSI with concentrations different astaxanthin in diets; b) HSI in control; c) HSI with concentrations different β-carotene in diets; d) GSI and astaxanthin;(e) GSI and control; f) GSI and β-carotene.

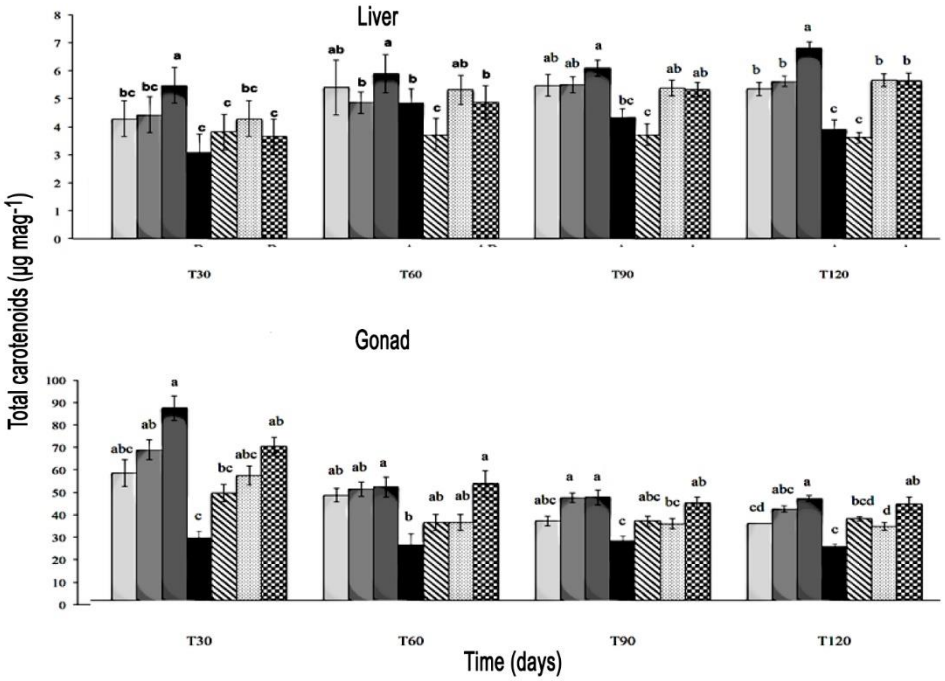

Figure 3. Total carotenoid concentrations (µg mg^{-1}) in the liver and gonads of the goldfish *C. auratus*. Lowercase and uppercase letters show the ANOVA analysis and subsequent Tukey's HSD *post-hoc* test ($P < 0.05$) for treatments in each time sampling and for each treatment between sampling times, respectively.

Table 1. Correlation between HSI index and GSI index with total carotenoids in the ovary and liver for 120 days and 84 ind. HIS: Hepatosomatic Index, GSI: Gonadosomatic Index, ns: non-significant, *Significant at level $P < 0.05$.

	HIS	GSI	Ovary total carotenoids	Liver total carotenoids
HSI	1			
GSI	0.20^{ns}	1		
Ovary carotenoids	0.12^{ns}	0.36*	1	
Liver carotenoids	-0.17^{ns}	0.31*	0.38*	1

are presented in Table 1. The analysis of total carotenoid concentrations in the liver and the gonads showed a positive correlation ($P < 0.05$). Total carotenoids in the liver showed a significant positive correlation with the GSI ($P < 0.05$).

DISCUSSION

Carotenoids present in the diet, when consumed by fish, accumulate in the liver before being transferred to the ovaries in the late stages of maturity (Tiskar *et al.*, 2013). β-carotene consumed by female spawners remains in the liver, where it is transformed into vitamin A1 and subsequently vitamin A2 in *C. auratus*, which is then transported later on to the eggs during yolk formation (Del Tito, 1983). Previous studies that examined the effects of carotenoid supplementation in rainbow trout, gilthead seabream and yellow tail cichlid broodstock (Lakeh *et al.*, 2010; Scabini *et al.*, 2011; Güroy *et al.*, 2012) and carp (Tizkar *et al.*, 2013) diets have been undertaken. Our results show that dietary supplementation of carotenoids (astaxanthin and β-carotene) in female goldfish increased the GSI but did not affect the HSI. This result indicated that at low dietary levels, most β-carotene consumed by goldfish was utilized for its biological roles thus the HSI not increase significantly, as reported in this study. At high dietary β-carotene concentrations, unutilized vitamin A can be stored in the liver, resulting in a significant increase of HSI. This has also been observed in hybrid tilapia (Hu *et al.*, 2006). The pattern of carotenoid utilization between the gonads and the liver is in agreement with the results of similar studies with other fish (Sadekarpawar & Parikh, 2013). The inverse relationship between the HSI and the GSI shows a continuous transfer of energy reserve from the liver to the gonads (Izquierdo *et al.*, 2001). The GSI and HSI indices are useful indicators of the effects of exposure

to different substances, growth promoters and carotenoids in fish.

Sumpter & Jobling (1995) found that the livers of fish, particularly that of females, contains high concentrations of estrogen receptors, which accounts for its ability to synthesize large amounts of vitellogenin when stimulated by estrogen and carotenoids (Palace & Werner *et al*., 2006). The carotenoid enriched diets produced a positive effect on goldfish GSI and could lead to an increase in gonad weight broodstock (Chainapong & Traichaiyaporn, 2013). In salmonids, carotenoids are absorbed in the intestine, transported into the blood attached to the surface of the chylomicron spheres, metabolized in the liver, and stored in muscle tissue, then transported from the muscle to the skin and the ovaries (Rajasingh *et al*., 2006). Other marine fish species accumulate carotenoids in their skin, eggs and liver rather than in muscle tissues (Bjerkeng & Berge, 2000).

In the present study, carotenoid levels in the liver did not change during the conditioning period except for the control and β-carotene diets (B150), which were lower in the beginning of the experiment. However, at days 90 and 120 of the experiment, the livers of fish in the A150 treatment showed high carotenoid levels. These findings could be related to the accumulation of carotenoids in other fish tissues over the conditioning period. According to Rajasingh *et al*. (2006), in salmonids the astaxanthin absorbed by the intestine is metabolized in the liver and stored in the skin and gonads during maturation and in muscle tissue during growth. The results of our study reflect similar trends, as demonstrated by the positive correlation between liver and ovary carotenoid levels at the beginning and at the end of the experiment. The liver works as a metabolic and excretory organ for carotenoids (Torrissen & Ingebrigtsen, 1992).

Carotenoid levels in the gonads were higher for astaxanthin-enriched diets (A100 and A150) and for the β-carotene diet (B150). Higher carotenoid levels were noted for both astaxanthin and β-carotene treatments at the beginning of the conditioning period. Ando *et al*. (1986) suggest that carotenoids are transported from the muscles to the gonads by a carotenoid-carrying lipoprotein called vitellogenin (a female-specific serum lipoprotein produced by the liver) during spawning migration, and accumulate in egg yolk (where they bind to lipovitellin). The intensity of this transition is higher in the initial stages of sexual maturation (Foss *et al*., 1984), as observed in the present study.

For rainbow trout, research shows that increased levels of astaxanthin boost glycogen levels during sexual maturation, which may improve liver function and enhance the performance of other nutrients in converting intermediate substance (Metusalach *et al*., 1996).

In conclusion, diets enriched with 150 mg kg^{-1} of astaxanthin and β-carotene improved the sexual maturation of goldfish broodstock and increased gonadosomatic index (GSI). Supplementing the diet of goldfish with astaxanthin had a significant inverse relationship ($P < 0.05$) between the values and pigment concentration of the GSI and the HSI. These indices can also be used as a reliable indicator of the reproduction conditions of *C. auratus* female broodstock.

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REFERENCES

Ando, S., F. Yamazaki & M. Hatano. 1986. Effect of 17a-methyltestosterone on muscle composition of chum salmon. Bull. Jap. Soc. Sci. Fish., 52: 565-571.

Association of Official Analytical Chemists (AOAC). 2000. Official methods of analysis of AOAC International. Association of Official Analytical Chemists, Gaithersburg, 2200 pp.

Barber, I., S.A. Arnott, V.A. Braithwaite, J. Andrew, W. Mullen & F.A. Huntingford. 2000. Carotenoid-based sexual coloration and body condition in nesting male sticklebacks. J. Fish Biol., 57: 777-790.

Barber, B.J. & N.J. Blake. 2006. Reproductive physiology. In: S.E. Shumway & G.J. Parsons (eds.). Scallops: biology, ecology, and aquaculture. Elsevier, Amsterdam, pp. 357-416.

Bjerkeng, B. & G.M. Berge. 2000. Apparent digestibility coefficients and accumulation of astaxanthin E/Z isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp. Biochem. Physiol., 127(3): 423-432.

Chainapong, T. & S. Traichaiyaporn. 2013. Enhancement of carotenoid production in *Spirulina platensis* and fed on *Clarias macrocephalus* for reproductive performance. J. Agr. Tech., 9(1): 49-59.

Craik, J.C.A. 1985. Egg quality and egg pigment content in salmonid fishes. Aquaculture, 47: 61-88.

Del Tito, B.J. 1983: Role of beta-carotene and lutein in the synthesis of vitamin A in goldfish. Prog. Fish-Cult., 45(2): 94-97.

Diniz, M.S., I. Peres, I. Magalhães-Antoine, J. Falla & J.C. Pihan. 2005. Estrogenic effects in crucian carp (*Carassius carassius*) exposed to treated sewage effluent. Ecotoxicol. Environ. Saf., 62: 427-435.

Food and Agriculture Organization (FAO). 2013. Fisheries and aquaculture information and statistics service. [http://www.fao.org/fishery/topic/166291/en]. Reviewed: 10 November 2014.

Foss, P., T. Storebakken, K. Schiedt, S. Liaaen-Jensen, E. Austreng & K. Streiff. 1984. Carotenoids in diets for salmonids. 1. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. Aquaculture, 41: 213-226.

Güroy, B., I. Sahin, S. Mantoglu & S. Kayal. 2012. Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. Aquacult. Int., 20: 869-878.

Hu, C.J., S.M. Chen, C.H. Pan & C.H. Huang. 2006. Effects of dietary vitamin A or β-carotene concentrations on growth of juvenile tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquaculture, 253: 602-607.

Izquierdo, M. & H. Fernandez-Palacios. 1997. Nutritional requirements of marine fish larvae and broodstock. In: A.G.J. Tacon & B. Basurco. (eds.). Feeding tomorrow's fish. Cah. Opt. Medit., 22: 243-264.

Izquierdo, M.S., H. Fernandez-Palacios & A.G.J. Tacon. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, 197: 25-42.

Kerfeld, C.A., M.R. Sawaya, V. Brahmandam, D. Cascio, K.K. Ho, C.C. Trevithick-Sutton, D.W. Krogmann & T.O. Yeates. 2003. The crystal structure of a cyanobacterial water-soluble carotenoid binding protein. Structure, 11: 55-65.

Lakeh, A.A.B., M.R. Ahmadi, S. Safi, T. Ytrestøy & B. Bjerkeng. 2010. Growth performance, mortality and carotenoid pigmentation of fry offspring as affected by dietary supplementation of astaxanthin to female rainbow trout (*Oncorhynchus mykiss*) broodstock. J. Appl. Ichthyol., 26: 35-39.

Lee, J.S. & M.E. Newman. 1997. Aquaculture. Interstate Publishers, İllinois, 518 pp.

Levene, H. 1960. In contributions to probability and statistics: essays in Honor of Harold Hotelling. In: I. Olkin (ed.). Stanford University Press, Stanford, 278-292.

Maoka, T. 2011. Carotenoids in marine animals. Mar. Drugs, 9: 278-293.

Metusalach, S.J., J. Brown & F. Shahidi. 1996. Deposition and metabolism of dietary canthaxanthin in different organs of Arctic charr (*Salvelinus alpinus* L.). Aquaculture, 142: 99-106.

Page, G.I. & S.J. Davies. 2003. Hepatic carotenoid uptake in rainbow trout (*Oncorhynchus mykiss*) using an isolated organ perfusion model. Aquaculture, 225: 405-419.

Palace, V.P. & J. Werner. 2006. Vitamin A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Sci. Mar., 70(2): 41-57.

Rajasingh, H., L. Øyehaug, D.I. Våge, W. Stig & S.W. Omholt. 2006. Carotenoid dynamics in Atlantic salmon. B.M.C. Biology, 4: 1-15.

Sadekarpawar, S. & P. Parikh. 2013. Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. World J. Zool., 8(1): 110-118.

Sawanboonchun, J., W.J. Roy, D.A. Robertson & G. Bell. 2008. The impact of dietary supplementation with astaxanthin on egg quality in Atlantic cod broodstock (*Gadus morhua*, L.). Aquaculture, 283: 97-101.

Scabini, V., H. Fernandez-Palacios, L. Robaina, T. Kalinowski & M.S. Izquierdo. 2010. Reproductive performance of gilthead seabream (*Sparus aurata* L., 1758) fed two combined levels of carotenoids from paprika oleoresin and essential fatty acids. Aquacult. Nutr., 17: 304-312.

Sühnel, S., F. Lagreze, J.F. Ferreira, L.H. Campestrini & M. Maraschin. 2009. Carotenoid extraction from the gonad of the scallop *Nodipecten nodosus* (Bivalvia; Pectinidae). Braz. J. Biol., 69: 209-215.

Sumpter, J.P. & S. Jobling. 1995. Vitellogenesis as biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect., 103: 173-178.

Tizkar, B., M. Soudagar, M. Bahmani, S.A. Hosseini & M. Chamani. 2013. The effects of dietary supplementation of astaxanthin and β-caroten on the reproductive performance and egg quality of female goldfish (*Carassius auratus*). Caspian. J. Environ. Sci., 11(2): 217-231.

Torrissen, O.J. & K. Ingebrigtsen. 1992. Tissue distribution of 14C-astaxanthin in the Atlantic salmon (*Salmo salar*). Aquaculture, 108: 381-385.

Watanabe, T. & R. Vassallo-Agius. 2003. Broodstock nutrition research on marine finfish in Japan. Aquaculture, 227: 35-61.

Wooster, G.A. & P.R. Bowser. 2000. Remediation of Cayuga Syndrome in landlocked Atlantic salmon (*Salmo salar*) using egg and sac-fry bath treatments of thiamin-hydrochloride. J. World. Aquacult. Soc., 31: 149-157.

Yanar, M., Z. Erçen, A.Ö. Hunt & H.M. Büyükapar. 2008. The use of alfalfa, *Medicago sativa* as a natural carotenoid source in diets of goldfish, *Carassius auratus*. Aquaculture, 284: 196-200.

Yuangsoi, B., O. Jintasataporn, N. Areechon & P. Tabthipwon. 2010. The use of natural carotenoids and growth performance, skin pigmentation, and immune response in fancy carp (*Cyprinus carpio*). J. Appl. Aquacult., 22: 267-283.

Yuangsoi, B., O. Jintasataporn, N. Areechon & P. Tabthipwon. 2011. The pigmenting effect of different carotenoids on fancy carp (*Cyprinus carpio*). Aquacult. Nutr., 17(2): e306-e316.

Zar, J.H. 2010. Biostatistical analysis. Prentice Hall, New Jersey, 944 pp.

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