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RESEARCH PAPER

Evaluation of Natural and Synthetic Carotenoid Supplementation on Growth, Survival, Total Carotenoid Content, Fatty Acids Profile and Stress Resistance of European Seabass, *Dicentrarchus labrax*, Fry

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Abstract

A feeding experiment was conducted to evaluate the effects of different levels of dietary carotenoid sources on growth, antioxidant responses, total carotenoid content, fatty acid profile and stress resistance of European seabass (*Dicentrarchus labrax*) with 0.3±0.02 g. Three types of carotenoids derived from either synthetic astaxanthin (AX; 0.05, 0.1 g kg^{-1}), or natural source: marigold flower meal (ML; 0.1, 0.2, 0.3 g kg^{-1}) and crab waste meal (CM; 5, 10, 15 g kg^{-1}) were separately tested. The control diet (CTR) had no carotenoid supplementation. Nine fish treatments (three replicates each) were fed isonitrogenous (47% CP) and isolipidic (12.5%) diets three times per day to apparent satiation for 10 weeks. The study indicated that dietary supplementation with CM10 and ML0.2 exhibited better growth performance, weight gain, daily growth index and growth coefficient than the control group. After 10 weeks, total body carotenoids content significantly ($P<0.05$) increased with increasing supplemented levels AX, ML and CM in fish diets. Meanwhile, thiobarbituric acid-reactive substances were significantly decreased in fish fed diets supplemented with high levels of carotenoid sources. Furthermore, carotenoids supplementation significantly improved fish resistance to thermal and salinity stress compared with control fish ($P>0.05$). Results have suggested that dietary inclusion of CM and ML at doses of 10 or 0.2 g kg^{-1} respectively, could improve growth, total carotenoid content, antioxidant activities and stress resistance of seabass fry as efficiently as the commercial synthetic astaxanthin.

Introduction

European seabass (*Dicentrarchus labrax*), is considered to be the most important marine fish currently cultured in the Mediterranean Sea and other waters (Assem, 2004). The most important producers in the Mediterranean areas are Turkey, Greece, Spain, Italy, Egypt and Croatia. Annual production was more than 134 978 tons in 2012 (FEAP, 2014). Meanwhile the total Egyptian production of Seabass was 16447 tons contributing 1.11 % of the total production (GAFRD, 2015).

In aquaculture, fish feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both growth efficiency and feed wastage (El-Sayed, 2004)

Within fish feeds industry, fat-soluble pigments such as carotenoids are required for optimal nutritional requirements and coloration of fish (NRC 2011). Carotenoids can also improve survival rate, metabolism, reproduction and enhance resistance to several stress conditions as well as pigmentation of fish (Amar, Kiron, Akutsu, Satoh, & Takeshi, 2012; Wade *et al*. 2015; Zelaty, Murthy, Nazarkardeh,

Ahmadiyan & Meshram, 2016; Karadal, Güroy, & Türkmen, 2017) and further keep the harmful impacts of lipid peroxidation (Waagbo *et al*., 2003).

It is known that, fishes have no access to carotenoids in captive culture system and therefore, the necessary carotenoids must be added to the diet (Torrissen, Christiansen, Struknæs, & Estermann, 1995). The natural carotenoid sources have been obtained from specific sources, such as crustaceans and plants (Gupta, Jha, Pal, & Venkateshwarlu, 2007). However, natural and synthetic carotenoids are usually added to commercial feeds for improving pigmentation of the skin and muscle flesh (Karadal *et al*., 2017). However, the addition of synthetic carotenoids such as astaxanthin is expensive and led to increase feed production costs and therefore, reduced profits of the aquaculture process (Choubert, Cravedi, & Laurentie, 2009).

Previous studies carried out by Kalinowski, Izquierdo, Schuchardt, & Robaina, 2007; Lee and Lee 2008 and Hynes, Egeland, Koppe, Baardsen, & Kiron, (2009) have tended to add alternative carotenoid sources for aquafeeds, including natural carotenoids. Marigold flower (*Tagetes erecta*) is one of the most important sources of these type of carotenoids which contains about 7000 mg kg^{-1} lutein and zeaxanthin, in addition to being a source of nutrients such as protein, fat and other vitamins (Navarrete-Bolanos, Rangel-Cruz, Jimenez-Islas, Botello-Alvarez, & Rico-Martinez, 2005; Del Villar-Martinez *et al*., 2007). Lutein is the main active components in marigold flowers extract which is the most common carotenoids widely used in aquafeeds to improve the survival of fish juvenile stages by strengthening their immunological system (Amar *et al*., 2012).

Crustaceans such as, Atlantic krill, crayfish, crab, etc., and some marine microorganisms are rich source of astaxanthin and used as additives in aquafeeds (Kalinowski *et al*., 2007; Kurnia *et al*., 2015).

This study aims to evaluate the effects of different carotenoids source, namely: synthetic astaxanthin, marigold (*Tagetes erecta*) flower meal and crab (*Portunus pelagicus*) waste meal in the diet of seabass *Dicentrarchus labrax* fry on growth, feed utilization, carotenoid content of tissues and antioxidant activity.

Materials and Methods

Experimental Fish and Rearing Conditions

Five hundred and forty European seabass (*Dicentrarchus labrax*) fry (average weight of 0.3±0.02 g and average length with 3.3±0.02 cm) were obtained from the Marine Hatchery of National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Fish were acclimatized to the indoor rearing conditions and fed the control diet for two weeks before the beginning of the experiment in Marine Fish Nutrition Laboratory, NIOF, Alexandria, Egypt. Fish were randomly assigned into nine experimental treatments with three replicates per treatment at a stocking density of 20 fish per aquarium. Experimental aquariums (60 liters) were filled with aerated and filtered saline water from an underground well passed through an 80 µm filter and a sand filter. The rearing conditions were follow: temperature (22 ± 1.2 °C), salinity (32 ± 0.16 g L^{-1}), pH (7.8 ± 0.1) and dissolved oxygen (6.81 ± 0.2 mg L^{-1}) throughout the feeding trial. Fish were held under a natural photoperiod of approximately (12:12 h light:dark). The feeding frequency was three times daily at 09:00, 12:00 and 15:00 hours for 10 weeks. To prevent the waste of pellets, fish were slowly hand-fed to satiation based on visual observation of their feeding behavior. Feed consumption was recorded for each aquarium every day. During the period of feeding, Feces were cleaned manually before first feeding by siphoning and 30% of water in fish aquarium was exchanged daily.

At the end of the feeding trial, all fish in each aquarium were collectively weighed after anesthetizing with 0.6 mL clove oil (Cairo Pharmaceutical Co., Alexandria, Egypt), after starvation for 24 h to calculate growth performance, feed utilization and indices. Three fish from each aquarium (n=9 per treatment) were sampled to calculate condition factor, hepatosomatic and visceral somatic indices. Mention that fish length was measured, liver and intestine were carefully removed from dissected-out fish and accurately weighed for hepatosomatic and visceral somatic indices.

Experimental Design and Diets

Nine experimental diets were formulated by using locally available feed ingredients to contain 47% crude protein and 12.5% crude lipid. Three carotenoid sources either synthetic, e.g. astaxanthin (AX), or natural e.g. marigold petal meal (ML) and crab waste meal (CM), were tested. The control, diet 1(CTR) had no added carotenoids, whereas diets from 2 to 9 contained a different level of a carotenoid source. Diets 2&3 contained astaxanthin (AX) at 2 levels, 0.05 and 0.1 g kg^{-1}, and designated as: AX0.05, AX0.1. Diets 4-6 contained marigold meal (ML) at 3 levels: 0.1, 0.2, 0.3 g kg^{-1} and designated as; ML0.1, ML0.2, ML0.3 and diets 7-9 contained crab-waste meal (CM) at 3 levels: 5, 10, 15 g $kg^{-}1$ and designated as; CM5, CM10, CM15 respectively.

All dry ingredients were finely ground in a hammer mill and passed through a 250-µm mesh

sieve and weighed according to the composition of diet and mixed mechanically by a feed mixer (Damai HC-1500A, Zhejiang, China). Micro components were premixed first than added to the dry mix of feed ingredients. Oil sources (including fish oil and soybean oil) were added last to the dry mixture and thoroughly mixed. Warm distilled water (150 mL kg^{-1}) was then added to the mixer to produce moist dough. For pelleting, the dough was screw-pressed through an electric meat mincer machine (Tornado MG-2000, Egypt) fitted with 1 mm diameter die. After that, the diets were dried in a ventilated oven at 40°C until the moisture below 100 g/kg, and all diets were stored at -20°C until use. The proximate composition and carotenoid concentration of the experimental diets are given in Table 1.

Carotenoid Sources

Astaxanthin (AX): (CAROPHYLL®) containing 10% synthetic astaxanthin powder made by Xi'an Quanao Biotech Co., Ltd. And was purchased from Trust Care Company for trading, Egypt. The price is 460 US $/Kg

Crab meals (CM): Crabs were collected from the local market, and then meal was prepared from shells using autoclave at a high pressure for 10 min, then oven-dried for 12 h at 5 °C and finally ground in a hammer mill through a 250-µm mesh. Marigold meal (ML): Fresh marigold flowers were collected from Alexandria city, Egypt**.** The petals were separated and dried at 40-50 °C for 24 h. All supplementations were weighed and mixed carefully into oil and then added to control diet with each respective level according to Wade *et al*. (2015) and Sánchez-Martínez *et al*. (2015).

Evaluating the Growth Performance and Nutrient Utilization

The final mean body weight (FBW) of each experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish, then calculate average for three replicates. Weight gain (WG), length gain (LG), daily growth index (DGI), growth coefficient (GC), feed conversion ratio (FCR), protein efficiency ratio (PER), protein

Table 1. Feed ingredients and proximate composition of the experimental diets (g kg^{-1})

	Diets (g kg^{-1})[1]								
	CTR	$SA_{0.05}$	$SA_{0.1}$	$ML_{0.1}$	$ML_{0.2}$	$ML_{0.3}$	CM_5	CM_{10}	CM_{15}
Ingredients (g kg^{-1})									
Fish meal [2]	400	400	400	400	400	400	400	400	400
Soybean meal [2]	310	310	310	310	310	310	310	310	310
Maize gluten meal	61	61	61	61	61	61	61	61	61
Wheat flour	110	110	110	110	110	110	105	100	95
Fish oil	80	80	80	80	80	80	80	80	80
Soybean oil	20	20	20	20	20	20	20	20	20
$Ca(H_2PO_4)_2$	7	7	7	7	7	7	7	7	7
Vitamin and mineral mix.[3]	3	3	3	3	3	3	3	3	3
CMC (binder)[4]	6	6	6	6	6	6	6	6	6
Choline chloride	1	1	1	1	1	1	1	1	1
L-lysine (51%)	1	1	1	1	1	1	1	1	1
DL-methionine (99%)	1	1	1	1	1	1	1	1	1
Added carotenoids (g kg^{-1})	0	0.05	0.1	0.1	0.2	0.3	5	10	15
Proximate composition (g kg^{-1}) on dry matter basis:									
Moisture	89	88	87	89	79	71	83	88	78
Crude protein	474	475	471	471	472	477	475	481	477
Crude lipid	126	125	126	124	125	124	126	126	126
Ash	94	95	94	93	94	95	96	97	98
Gross energy (KJ g^{-1}) [5]	21.0	20.9	20.9	20.9	20.9	20.9	20.9	21.0	20.8
Total carotenoids (µg g^{-1})	18	196	322	348	360	403	272	287	300

[1] SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal. Control diet (CTR); 0.05 g SA kg^{-1} ($SA_{0.05}$); 0.1 g SA kg^{-1} ($SA_{0.1}$); 0.1 g ML kg^{-1} ($ML_{0.1}$); 0.2 g ML kg^{-1} ($ML_{0.2}$); 0.3 g ML kg^{-1} ($ML_{0.3}$); 5 g CM kg^{-1} (CM_5); 10 g CM kg^{-1} (CM_{10}) and 15 g CM kg^{-1} (CM_{15}).

[2] Fish meal (Peru steam-treated), crude protein 675 g kg-1 dry matter, crude lipid 92 g kg^{-1} dry matter; soybean meal (solvent extracted), crude protein 480 g kg^{-1} dry matter.

[3] Vitamin and mineral mix contains (mg kg^{-1}) E, 30; K, 3; thiamine, 2; riboflavin, 7; pyridoxine, 3; pantothenic acid, 18; niacin, 40; folacin, 1.5; choline, 600; biotin, 0.7; cyanocobalamin, 0.02; Mg, 100; Zn, 60; Fe, 40; Cu, 5; Co, 0.1; I, 0.1 and BHT, 100.

[4] CMC: Henan Jianjie Shiye Co., Ltd. (Zhengzhou, China).

[5] GE: gross energy content calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g^{-1} protein, ether extract and NFE respectively (NRC, 1993).

productive value (PPV), energy retention (ER) and survival (%) were calculated according to Xue *et al*. (2006) by using the following equations:

Weight gain (WG)= final body weight (g)-initial body weight (g)

Daily growth index (DGI) = $100 \times (W_f^{1/3} - W_i^{1/3}/\text{days})$

Growth coefficient (GC)= $100 \times (W_f^{1/3} - W_i^{1/3}/ \Sigma\theta)$

where W_f= final weight (g), W_i= initial Weight (g), $\Sigma\theta$= sum of average daily temperature in °C.

Length gain (LG) = final length (cm)-initial length (cm)

Condition factor (CF)=$100 \times (FW/L^3)$

where: FW= fish weight (g), L= total fish length (cm).

Survival rate (%) = 100 × (final fish number / initial fish number)

Feed conversion ratio (FCR)= feed intake (g)/weight gain (g)

Protein efficiency ratio (PER)= weight gain (g)/protein intake (g)

Protein productive value (PPV; %)= protein gain (g)/protein intake (g) × 100

Energy retention (ER; %)= energy gain (kJ)/energy intake (kJ) × 100

Evaluating the Body Indices

The body indices (%) were calculated as % of body weight as mentioned afterward:

Hepato-somatic index (HIS) = 100 × [Liver weight (g)/body weight (g)]

Viscera-somatic index (VSI) = 100 × [Viscera weight (g)/body weight (g)]

Intestine length index (ILI) =100 × [intestine length (cm)/body length (cm)]

Whole Body Chemical Composition

Fish samples were collected at the beginning and the end of the experiment and stored at -20 °C for proximate chemical analysis according to AOAC (2000). The samples of experimental diets were dried to constant weight at 105 °C in a drying oven to determine moisture content. Crude protein was determined by digestion using the micro-Kjeldahl method (Kjeltec, Model VELP Scientifica, UDK 127, Usmate, Italy) and estimated by multiplying *N* by 6.25. Crude lipid was measured by ether extraction using the Soxhlet method (Model VELP Scientifica, SER 148). Ash was determined by muffle furnace at 550 °C for 4 h.

Determination of Fatty Acids Profile

The fatty acid composition was determined on fish muscles. The lipid extraction of the wet samples was performed according to Hara and Radin (1978) and the transmethylation of the fatty acid according to Christie (1982) with the modifications described by Chouinard, Corneau, Sæbø, & Bauman, 1999. The fatty acid methyl esters in hexane were then injected into a gas chromatograph (GC 1000 DPC, Dani Instruments S.P.A., Cologne Monzese, Italy) equipped with a flame ionization detector. The separation of the fatty acid methyl esters was performed using a Famewax™ fused silica capillary column (30 m×0.25 mm (i.d.), 0.25 µm; Restek Corporation, Bellefonte, PA, USA). The peak area was measured using a Dani Data Station DDS 1000. Each peak was identified and quantified by pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

Total Body Carotenoid Analysis

Total carotenoid content (TCC) in a homogenate of whole fish body and the tested diets were analyzed according to the method described by Olson (1979) using three pooled samples per treatment. Briefly, two fish for each replicate were anaesthetized with t-amyl alcohol, and 1 g of whole body (without head and alimentary canal) was taken in a 10 ml screw capped clear glass vials and 2.5 g of anhydrous sodium sulphate was added. The sample was gently meshed with a glass rod against the side of the vial and then 5 mL of Chloroform was added and left overnight at 0 °C. When the chloroform formed a clear 1-2 cm layer above the caked residue, the optical density was read at 380, 450, 470 and 500 nm, in a spectrophotometer (Spectrophotometer PD-303 UV, APEL, Kawaguchi, Japan), taking 0.3 mL aliquots of chloroform diluted to 3 mL with absolute ethanol. A blank prepared in a similar manner was used for comparison. The wave length, at which maximum absorption, was used for the calculation:

Total carotenoid content = [absorption at maximum wavelength /(0.25 x sample weight (g)] x 10

Where, 10 = Dilution factor; 0.25 = Extinction coefficient

Antioxidant Capacity Analysis

Samples were washed using sterile chilled saline, minced and homogenized (10% w/v) in ice-cold sucrose buffer (0.25 M) in a Wise Tis® HG-15D homogenizer (Daihan Scientific, Bangalore, India). The resulting supernatant was collected after centrifugation at 7063 rpm, 4°C for 20 min and used for antioxidant enzyme assays.

Thiobarbituric acid-reactive substances (TBARs) were measured in homogenates at 532 nm using 2-thiobarbituric acid (TBA; 2-thioxodihydropyrimidine-4,6). The principle of the procedure is that, at low pH and elevated temperature, malondialdehyde (MDA; end product of lipid peroxidation) readily participates in a nucleophilic addition reaction with TBA to form MDA:TBA adducts (1:2), generating red fluorescence. The color intensity is proportionate to MDA levels. An extinction coefficient of 156 000 M -1 cm -1 was used for the calculation (Tappel and Zalkin 1959).

Total antioxidant capacity (TAC) was assayed using the method of Cao, Verdon, Wu, Wang, & Prior, 1995. Briefly, the total antioxidant capacity was measured by the reaction of an antioxidant in 0.02 mL of homogenate with 0.5 mL of hydrogen peroxide (H_2O_2), incubated for 10 min at 37 °C. The antioxidants in the sample eliminated a certain amount of the H_2O_2 provided. The residual H_2O_2 was determined calorimetrically by an enzymatic reaction which involved the conversion of 3,5,dichloro-2-hydroxybenzene sulphonate into a colored product assessed at 505 nm.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using the method of the International Federation of Clinical Chemistry (Gella *et al*., 1985). The principle reaction of the colorimetric determination of AST or ALT activity is based on the reaction of aspartate or alanine with a-ketoglutaric acid to form oxaloacetate or pyruvate respectively. The oxaloacetate or pyruvate was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The absorbance is read at a wavelength of 505 nm. The absorbance was then interpolated in the calibration curve.

Stress Tolerance

At the end of the experiment, fish were kept in three aquariums with dimensions of (35x30x40cm; 35 L) at the same rearing condition. Fish were challenges by thermal stress (33 °C), low salinity stress (10 ppt) and combination of thermal stress and low salinity stress (33 °C plus 10 ppt). The challenges were done for 5 h and then return to normal rearing conditions. These processes were carried out for 5 consecutive days. Mortality was daily recorded in each group for 13 days. This protocol was conducted for control and treated fish according to Chien, Pan & Hunter (2003).

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Michigan Avenue, Chicago, IL, USA). Data were expressed as mean ± SD and subjected to a one-way ANOVA followed by Duncan's multiple range test (Duncan 1955). Significant differences were set $P<0.05$. All percentage data were arc-sign transformed prior to analysis (Zar, 1984).

Results

Growth Performance

Growth performance and survival (%) of

Table 2. Growth performance and survival (%) of European seabass, *Dicentrarchus labrax,* fed the experimental diets for 10 weeks

Dietary treatment	Parameter*						
	Final body weight (g fish^{-1})	Weight gain (g fish^{-1})	Daily growth index	Growth coefficient (%)	Length gain (cm)	Condition factor	Survival (%)
CTR	2.07±0.01^{e}	1.78± 0.01^{e}	0.84±0.01^{e}	2.69±0.00^{d}	2.27±0.23^{ab}	1.09±0.06	65.0± 2.89^{d}
$SA_{0.05}$	2.12±0.03^{e}	1.82±0.02^{e}	0.87±0.01^{de}	2.76±0.00^{d}	2.23±0.13^{ab}	1.18±0.10	68.3±1.67^{cd}
$SA_{0.1}$	2.29±0.03^{c}	1.97±0.03^{c}	0.94±0.02^{c}	2.99±0.01^{c}	2.83±0.09^{a}	0.99±0.04	75.0±2.89^{abc}
$ML_{0.1}$	2.21±0.01^{cd}	1.91±0.02^{cd}	0.91±0.01^{cd}	2.90±0.00^{c}	2.13±0.13^{ab}	1.18±0.09	65.0±2.89^{d}
$ML_{0.2}$	2.29±0.03^{c}	1.99±0.02^{c}	0.94±0.01^{c}	3.01±0.01^{c}	2.73±0.20^{ab}	1.03±0.11	78.3±3.33^{ab}
$ML_{0.3}$	2.16±0.02^{de}	1.84±0.02^{de}	0.88±0.01^{de}	2.79±0.00^{d}	2.00±0.10^{b}	1.30±0.04	70.0±2.89^{bcd}
CM_5	2.23±0.04^{cd}	1.94±0.03^{c}	0.93±0.01^{c}	2.94±0.01^{c}	2.27±0.50^{ab}	1.31±0.24	66.7±3.33^{cd}
CM_{10}	2.55±0.04^{a}	2.26±0.04^{a}	1.07±0.02^{a}	3.42±0.01^{a}	2.50±0.06^{ab}	1.24±0.06	81.7±1.67^{a}
CM_{15}	2.45±0.04^{b}	2.17±0.04^{b}	1.03±0.02^{b}	3.28±0.01^{b}	2.27±0.34^{ab}	1.31±0.11	75.0±2.89^{abc}

*Values are mean ± SE of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).

CTR, control diet; SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal; $SA_{0.05}$: 0.05 g SA kg^{-1}; $SA_{0.1}$: 0.1 g SA kg^{-1}; $ML_{0.1}$: 0.1 g ML kg^{-1}; $ML_{0.2}$: 0.2 g ML kg^{-1}; $ML_{0.3}$: 0.3 g ML kg^{-1}; CM_5: 5 g CM kg^{-1}; CM_{10}: 10 g CM kg^{-1} and CM_{15}: 15 g CM kg^{-1}.

Dicentrarchus labrax fry fed different levels of natural and synthetics carotenoids supplemented diets for 10 weeks were presented in Table 2. Results showed that the addition of different carotenoids to the diets significantly improved growth performance (FBW, WG, DGI and GC) and survival (%) of seabass compared to the control group (P<0.05). The highest values of GC reported in fry fed CM10 followed by CM15 then ML0.2 diets by 27.14, 21.93 and 11.90 respectively than the control diet. The survival (%) of all experimental groups ranged between 65 to 82%. The highest survival rates (%) were recorded with CM10 followed by ML0.2 which surpass the control group by 16.70 and 13.30%, respectively. Also, condition factor did not show any significant differences among studied groups, and no specific signs of disease were observed (P>0.05). These indicated that the superiority of natural carotenoids sources on synthetic AX.

Feed and Nutrient Utilization

All diets were accepted equally well by the fish. Also, the diets supplemented with different carotenoids sources were more acceptable than control diets, which showed an increasing trend of feed intake compared to the control diet after 10 weeks of the feeding trial (Table 3). The feed and nutrient utilization showed a significant improvement in FCR (g) and PER (g), in groups fed CM10 and CM15 compared to the control. Moreover, PPV (%) and ER (%) was increased significantly with high doses of different supplemented carotenoids (AX0.1, ML0.2, CM10 and CM15) compared to the control (Table 3).

Table 3. Feed and nutrients utilization of European seabass, *Dicentrarchus labrax,* fed the experimental diets for 10 weeks

Dietary treatment	Parameter*				
	Feed intake (g fish$^{-1)}$	Feed conversion ratio (g)	Protein efficiency ratio (g)	Protein productive value (%)	Energy retention (%)$^{\text{※}}$
CTR	7.91± 0.26	4.46± 0.17^{ab}	0.47±0.02^{bc}	6.91±0.34^{d}	2.63±0.06^{d}
$SA_{0.05}$	7.75± 0.23	4.26± 0.07^{abc}	0.50±0.01^{bc}	7.40±0.08^{bcd}	2.71±0.08^{cd}
$SA_{0.1}$	8.12± 0.02	4.13± 0.07^{bc}	0.51±0.01^{bc}	7.81±0.23^{bc}	3.06±0.10^{b}
$ML_{0.1}$	8.04 ± 0.22	4.20 ± 0.08^{abc}	0.51±0.01^{bc}	7.37±0.15^{bcd}	2.71±0.03^{cd}
$ML_{0.2}$	8.22± 0.07	4.14± 0.07^{bc}	0.51±0.01^{bc}	7.73±0.13^{bc}	2.88±0.01^{c}
$ML_{0.3}$	8.36 ± 0.17	4.53 ± 0.13^{a}	0.46±0.01^{c}	7.03±0.19^{cd}	2.64±0.05^{d}
CM_5	7.82± 0.25	4.03± 0.11^{cd}	0.52±0.02^{b}	7.99±0.37^{b}	2.70±0.04^{cd}
CM_{10}	8.07 ± 0.09	3.58 ± 0.08^{e}	0.59±0.01^{a}	9.10±0.21^{a}	3.54±0.06^{a}
CM_{15}	8.00± 0.32	3.70± 0.19^{de}	0.57±0.03^{a}	8.74±0.36^{a}	3.13±0.04^{b}

*Values are mean ± SE of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).
CTR, control diet; SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal; $SA_{0.05}$: 0.05 g SA kg^{-1}; $SA_{0.1}$: 0.1 g SA kg^{-1}; $ML_{0.1}$: 0.1 g ML kg^{-1}; $ML_{0.2}$: 0.2 g ML kg^{-1}; $ML_{0.3}$: 0.3 g ML kg^{-1}; CM_5: 5 g CM kg^{-1}; CM_{10}: 10 g CM kg^{-1} and CM_{15}: 15 g CM kg^{-1}.
※GE: gross energy content calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g $^{-1}$ protein, ether extract and NFE respectively (NRC, 1993).

Table 4. Proximate composition (% wet weight) and somatic indices of European seabass, *Dicentrarchus labrax*, fed the experimental diets for 10 weeks

Dietary treatment	Parameter*						
	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Gross energy (kJg^{-1})	Visceral somatic index (%)※	Hepatosomatic index (%)
CTR	70.62±0.59	14.32±0.46	7.06±0.11^{ab}	7.80±0.03^{a}	6.16±0.14^{bc}	9.08±0.31^{ab}	2.06±0.28^{ab}
$SA_{0.05}$	71.40±0.27	14.68±0.30	6.91±0.13^{b}	6.73±0.05^{de}	6.19±0.08^{bc}	6.20±0.49^{c}	1.24±0.09^{b}
$SA_{0.1}$	70.88±0.63	14.86±0.30	7.36±0.22^{a}	6.41±0.18^{e}	6.41±0.16^{ab}	8.27±0.54^{b}	1.81±0.42^{ab}
$ML_{0.1}$	71.59±0.21	14.34±0.10	6.44±0.02^{c}	7.19±0.15^{bc}	5.92±0.02^{cd}	9.17±0.80^{ab}	2.00±0.57^{ab}
$ML_{0.2}$	71.92±0.33	14.80±0.10	6.48±0.18^{c}	6.51±0.05^{e}	6.04±0.09^{cd}	8.10±0.21^{bc}	1.49±0.24^{b}
$ML_{0.3}$	71.23±0.14	14.88±0.20	6.24±0.11^{c}	7.31±0.05^{b}	5.97±0.08^{cd}	10.27±0.20^{ab}	2.27±0.14^{ab}
CM_5	72.35±0.35	14.95±0.26	5.83±0.02^{d}	6.49±0.13^{e}	5.82±0.07^{d}	9.65±0.33^{ab}	2.67±0.29^{a}
CM_{10}	71.08±0.20	15.21±0.19	7.33±0.09^{a}	6.10±0.08^{f}	6.48±0.06^{a}	10.99±1.24^{a}	2.08±0.19^{ab}
CM_{15}	71.26±0.28	15.10±0.29	6.26±0.12^{c}	6.97±0.08^{cd}	6.03±0.04^{cd}	9.51±0.91^{ab}	1.71±0.37^{ab}

*Values are mean ± SE of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).
CTR, control diet; SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal; $SA_{0.05}$: 0.05 g SA kg^{-1}; $SA_{0.1}$: 0.1 g SA kg^{-1}; $ML_{0.1}$: 0.1 g ML kg^{-1}; $ML_{0.2}$: 0.2 g ML kg^{-1}; $ML_{0.3}$: 0.3 g ML kg^{-1}; CM_5: 5 g CM kg^{-1}; CM_{10}: 10 g CM kg^{-1} and CM_{15}: 15 g CM kg^{-1}.
※GE: gross energy content calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g $^{-1}$ protein, ether extract and NFE respectively (NRC, 1993).

Whole-body Chemical Composition

Data on the whole-body composition are shown in Table 4. No significant (P<0.05) differences was recorded in moisture and protein (%) among all the experimental treatments. Crude lipid decreased with the higher dietary levels of all carotenoid supplementations, whereas, the lowest values was recorded for fish fed the ML0.3 and CM5 diets. Ash decreased significantly only with CM10 diet compared to other treatments. Meanwhile, the whole body gross energy was decreased significantly (P<0.05) with ML0.3 diet compared to control diet. The VSI and HSI did not differ significantly among carotenoids supplemented treatments and control except a reduction in the both parameters with AX0.05.

Antioxidant and Enzyme Activities

Table 5 given the changes in the levels of TBARs, TAC, AST and ALT in tissue of sea bass, fry fed the diet supplemented with different level of carotenoid sources. The results indicated that all experimental diets contain carotenoid significantly reduced tissue TBARs levels compared to the control diet. The lowest TBARs tissue values were recorded for fish fed the diets contain AX0.1 followed by AX0.05 and CM15. It is noticeable that, the effect of different levels of carotenoid on total antioxidant status (TAC) was level dependently. Moreover, CM and SA exhibit strong antioxidants more than ML. Meanwhile, fish fed control diet had the higher ALT and AST activities than all fish fed treated diet. Meanwhile, ALT and AST

Table 5. Thiobarbituric acid-reactive substances (TBARs), total antioxidant capacity, aspartate transaminase and alanine transaminase in wet tissue of European seabass, *Dicentrarchus labrax,* fed the experimental diets for 10 weeks

Dietary treatment	Parameter*			
	TBARs (nmol g^{-1})	Total antioxidant capacity (m mol g^{-1} w)	Aspartate transaminase (U g^{-1})	Alanine transaminase (U g^{-1})
CTR	88.28±0.85^{b}	32.50±0.50^{cd}	96.50±1.50^{a}	203.00±1.00^{a}
$SA_{0.05}$	72.11±0.19^{f}	31.00±1.00^{d}	61.50±1.50^{c}	169.50±1.50^{d}
$SA_{0.1}$	70.53±0.28^{f}	38.50±0.50^{c}	82.50±2.50^{b}	188.00±2.00^{bc}
$ML_{0.1}$	90.83±0.19^{a}	30.00±2.00^{d}	77.50±1.50^{b}	173.00±2.00^{d}
$ML_{0.2}$	78.89±0.85^{d}	37.50±1.50^{c}	95.50±6.50^{a}	198.00±2.00^{ab}
$ML_{0.3}$	86.98±0.96^{b}	38.00±1.00^{c}	80.50±0.50^{b}	180.00±3.00^{cd}
CM_{5}	82.87±0.46^{c}	35.00±2.00^{cd}	95.50±5.50^{a}	203.00±8.00^{a}
CM_{10}	79.16±0.33^{d}	71.00±1.00^{b}	37.50±0.50^{d}	156.00±1.00^{e}
CM_{15}	74.16±0.71^{e}	78.00±4.00^{a}	30.50±1.50^{d}	147.00±3.00^{e}

*Values are mean ± SE of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).
CTR, control diet; SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal; $SA_{0.05}$: 0.05 g SA kg^{-1}; $SA_{0.1}$: 0.1 g SA kg^{-1}; $ML_{0.1}$: 0.1 g ML kg^{-1}; $ML_{0.2}$: 0.2 g ML kg^{-1}; $ML_{0.3}$: 0.3 g ML kg^{-1}; CM_{5}: 5 g CM kg^{-1}; CM_{10}: 10 g CM kg^{-1} and CM_{15}: 15 g CM kg^{-1}

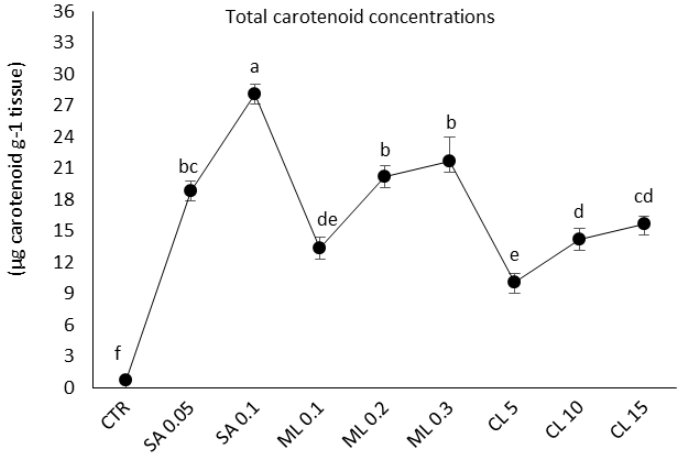

Figure 1. Total carotenoid concentrations in the tissue of European seabass, *Dicentrarchus labrax,* at the end of the feeding trial. SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal. Control diet (CTR); 0.05 g SA kg^{-1} ($SA_{0.05}$); 0.1 g SA kg^{-1} ($SA_{0.1}$); 0.1 g ML kg^{-1} ($ML_{0.1}$); 0.2 g ML kg^{-1} ($ML_{0.2}$); 0.3 g ML kg^{-1} ($ML_{0.3}$); 5 g CM kg^{-1} (CM_{5}); 10 g CM kg^{-1} (CM_{10}) and 15 g CM kg^{-1} (CM_{15}).

decreased with increasing dietary carotenoid concentrations. Whereas, the lowest ALT and AST value was recorded with fish fed diet contain CM15.

Total Carotenoids Content

The total carotenoids content in whole body of fish fed diets containing different levels of carotenoids were significantly (P<0.05) higher than that of fish fed the control diet (Figure 1). AX supplementation at level of 0.1 g kg^{-1} recorded the highest value of total carotenoid content (28.12 µg g^{-1}) in fish tissues followed by the diets of ML0.3 (21.63 µg g^{-1}), ML0.2 (20.13 µg g^{-1}) and CM15 (15.65 µg g^{-1}). Total carotenoid content significantly increased with increasing dietary carotenoid levels, regardless of the source of carotenoids.

Stress Tests

The effects of osmotic and thermal stress tests individual or in combination were presented in Figure 2. At the beginning of the different stress tests, the control fish exhibited remarkable decreased in the activity, which increased to higher activity after the first day. Also, hypoxia changes were associate as increasing of operculum movements with increasing random uncontrolled swimming interrupted by lapses into loss of equilibrium. Meanwhile, fish fed diets containing high level of AX, ML and CM showed slightly abnormal behavior due to different stressors. The main survival (%) of fish fed different carotenoid supplemented diets surpass fish in the control group at the end of stress tests. Also, regardless of the different carotenoid source the higher level increased fish tolerance to different conducted challenge tests (Figure 2).

Fatty Acids Profile

Fatty acids composition (FAs %) of fish samples, lipids of sea bass, fry fed different dietary carotenoid levels sources are presented in Table 6. Fish fed the diet contained any of the carotenoid sources had higher myristic acid C14:0 concentration in comparison with that of fish fed the control diet, and stearic acid C18:0 concentration significantly (P<0.05) increased in fish fed ML0.1 and ML0.2 diets.

Palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid n-6 recorded the highest significant (P<0.05) values in fish fed the diet of CM15 followed by CM10 and Ax0.1. Alpha linolenic acid (C18:3) FA in tissue of sea bass fry showed the highest significant (P<0.05) achieved by CM10 and the smallest significant (P<0.05) indicated by CM10 which recorded 1.28. The mead acid (C20:3) showed the significantly (P<0.05) highest exhibited by Ax0.1 followed by Ax0.05. The n-3 HUFA as total n-6 HUFA, EPA (C20:5) and DHA (C20:6) showed the highest significant (P<0.05) achieved by Ax0.1 and control diet, respectively.

Discussion

Several carotenoids are known to have a positive role in the intermediary metabolism of fish and crustaceans, improving growth and enhancing feed utilization (Amar *et al*., 2001 and Wade *et al*., 2015), in the current study, feeding European seabass natural carotenoids from marigold petals meal (ML) or crab waste meal (CM), has positive effects on growth or feed utilization as well as on survival. This was in line with a previous study with fry of European seabass, *Dicentrarchus labrax* where the growth, survival and feed utilization were positively affected

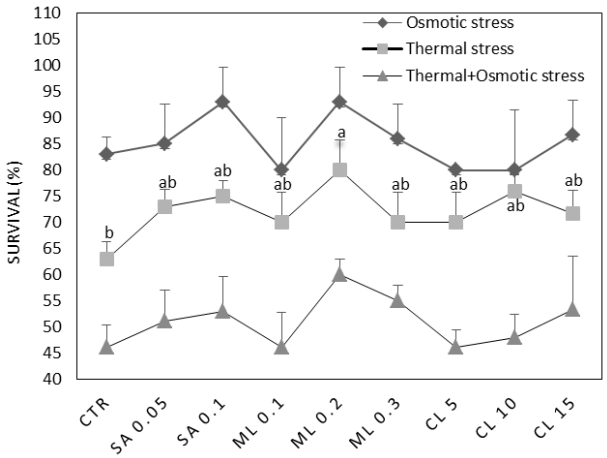

Figure 2. Stress tolerance on European seabass, *Dicentrarchus labrax,* at the end of the feeding trial. SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal. Control diet (CTR); 0.05 g SA kg^{-1} ($SA_{0.05}$); 0.1 g SA kg^{-1} ($SA_{0.1}$); 0.1 g ML kg^{-1} ($ML_{0.1}$); 0.2 g ML kg^{-1} ($ML_{0.2}$); 0.3 g ML kg^{-1} ($ML_{0.3}$); 5 g CM kg^{-1} (CM_5); 10 g CM kg^{-1} (CM_{10}) and 15 g CM kg^{-1} (CM_{15}).

Table 6. Fatty acid composition (% of total fatty acids) in the dorsal muscle of European seabass, *Dicentrarchus labrax,* fed the experimental diets for 10 weeks

Dietary treatment	CTR	$SA_{0.05}$	$SA_{0.1}$	$ML_{0.1}$	$ML_{0.2}$	$ML_{0.3}$	CM_5	CM_{10}	CM_{15}
C14:0	6.77 ^{b} ±0.58	8.02 ^{ab} ±0.50	6.89 ^{b} ±0.29	8.05 ^{ab} ±0.84	7.84 ^{ab} ±0.72	7.62 ^{ab} ±0.48	8.97 ^{a} ±0.18	8.92 ^{a} ±0.28	8.76 ^{a} ±0.25
C16:0	23.80 ^{bc} ±0.45	23.82 ^{bc} ±0.34	21.88 ^{d} ±0.13	26.43 ^{a} ±0.53	24.46 ^{b} ±0.42	22.79 ^{cd} ±0.34	25.06 ^{b} ±0.59	26.96 ^{a} ±0.25	27.24 ^{a} ±0.33
C18:0	5.99 ^{abc} ±0.28	4.26 ^{e} ±0.13	5.59 ^{bcd} ±0.43	7.11 ^{a} ±0.08	6.54 ^{ab} ±0.57	6.24 ^{abc} ±0.37	4.47 ^{de} ±0.31	5.07 ^{cde} ±0.37	5.98 ^{abc} ±0.33
C14:1	1.16 ^{c} ±0.05	1.17 ^{c} ±0.03	4.32 ^{a} ±0.60	3.17 ^{b} ±0.28	3.67 ^{ab} ±0.28	3.45 ^{b} ±0.25	0.42 ^{cd} ±0.01	0.00 ^{d} ±0.00	0.00 ^{d} ±0.00
C16:1	8.33 ^{bc} ±0.35	9.40 ^{b} ±0.49	7.19 ^{c} ±0.21	7.53 ^{c} ±0.40	7.35 ^{c} ±0.16	7.12 ^{c} ±0.22	12.21 ^{a} ±0.52	12.44 ^{a} ±0.49	12.47 ^{a} ±0.15
C18:1	14.92 ^{b} ±0.47	13.81 ^{bc} ±0.58	11.90 ^{e} ±0.12	14.15 ^{bc} ±0.26	13.25 ^{cd} ±0.26	12.36 ^{de} ±0.49	16.69 ^{a} ±0.48	17.16 ^{a} ±0.13	17.68 ^{a} ±0.13
C18:2	8.47 ^{bcd} ±0.16	8.78 ^{abcd} ±0.09	7.86 ^{d} ±0.44	8.07 ^{d} ±0.31	8.21 ^{cd} ±0.69	8.32 ^{bcd} ±0.14	9.69 ^{ab} ±0.61	9.47 ^{abc} ±0.47	10.07 ^{a} ±0.17
C18:3	0.82 ^{c} ±0.08	1.17 ^{ab} ±0.03	0.91 ^{bc} ±0.05	0.85 ^{c} ±0.05	0.94 ^{bc} ±0.18	1.01 ^{bc} ±0.07	1.16 ^{ab} ±0.02	1.28 ^{a} ±0.03	1.12 ^{ab} ±0.01
C20:3	8.44 ^{b} ±0.14	8.46 ^{b} ±0.08	9.63 ^{a} ±0.12	6.83 ^{d} ±0.38	7.91 ^{bc} ±0.16	6.64 ^{de} ±0.29	5.92 ^{e} ±0.29	7.37 ^{cd} ±0.02	8.02 ^{bc} ±0.32
C20:5	1.51 ^{ab} ±0.25	1.31 ^{ab} ±0.05	1.84 ^{a} ±0.34	1.61 ^{ab} ±0.22	1.73 ^{ab} ±0.37	1.82 ^{ab} ±0.06	1.04 ^{b} ±0.07	1.55 ^{ab} ±0.28	1.30 ^{ab} ±0.06
C22:6	10.03 ^{a} ±0.16	8.31 ^{b} ±0.41	9.81 ^{a} ±0.29	8.39 ^{b} ±0.13	9.09 ^{ab} ±0.79	8.88 ^{ab} ±0.23	3.84 ^{d} ±0.50	5.15 ^{c} ±0.25	6.01 ^{c} ±0.10
Total SFAs	38.38 ^{d} ±0.17	37.83 ^{d} ±0.37	35.79 ^{e} ±0.41	43.21 ^{ab} ±0.15	40.37 ^{c} ±0.47	38.18 ^{d} ±0.23	40.61 ^{c} ±0.10	42.86 ^{b} ±0.48	44.00 ^{a} ±0.29
n-3 HUFAs	30.02 ^{a} ±0.32	28.77 ^{ab} ±0.47	29.95 ^{a} ±0.67	26.55 ^{c} ±0.18	28.76 ^{ab} ±1.09	25.97 ^{c} ±0.14	22.69 ^{d} ±0.28	25.82 ^{c} ±0.23	27.31 ^{bc} ±0.10

*Values are mean ± SE of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).
CTR, control diet; SA, synthetic astaxanthin; ML, marigold petal meal; CM, crab waste meal; $SA_{0.05}$: 0.05 g SA kg^{-1}; $SA_{0.1}$: 0.1 g SA kg^{-1}; $ML_{0.1}$: 0.1 g ML kg^{-1}; $ML_{0.2}$: 0.2 g ML kg^{-1}; $ML_{0.3}$: 0.3 g ML kg^{-1}; CM_5: 5 g CM kg^{-1}; CM_{10}: 10 g CM kg^{-1} and CM_{15}: 15 g CM kg^{-1}.
SFAs, saturated fatty acids; HUFAs, highly unsaturated fatty acids.

by the addition of carotenoids (Sallam, Mansour, Srour, & Goda, 2017). The result revealed that the fish improved growth performance when diet was supplemented with ML which was similar with previous studies in red swordtail *Xiphophorus helleri* (Ezhil, Jeyanthi, & Narayanan 2008), golden pompano, *Trachinotus ovatus* (Xie *et al*., 2017) and yellowtail cichlid, *Pseudotropheus acei* (Guroy, Sahin, Mantoglu, & Kayali, 2012). Furthermore, the specific growth rate of koi carp, *Cyprinus carpio* significantly improved and decrease FCR even when 180 mg kg^{-1} marigold meal included as the dietary carotenoid source Swian, Ratnamanjari, Meshram, Mishra, & Shivananda (2014). Likewise, the growth rate of rainbow trout *Oncorhynchus mykiss* was positively influenced by dietary supplementation of marigold meal at level of 0.2 g kg^{-1} diet (Li *et al*., 2007). In addition, numerous studies have shown a negative effect of dietary carotenoid on growth and feed efficiency enhancement of gilthead seabream, *Sparus aurata* (Wassef, Chatzifotis, Sakr & Saleh 2010), rainbow trout, *Oncorhynchus mykiss* (Kurnia, Satoh, Haga, Kudo, Nakada, Matsumura, Watanabe, & Adachi, (015) and Atlantic salmon, *Salmo salar* (Buttle, 2001). These differences might be associated with different species, fish size, and feeding behavior. Meanwhile, the improvement in the performance parameters for the growth of European seabass fed with diets supplemented with carotenoid may be due to the main role of carotenoid as an antioxidant (Pan *et al*., 2010) and its involvement in intermediary metabolism (Talebi *et al*., 2013).

In the present study, the whole-body composition of European seabass was significantly affected by dietary natural carotenoid sources. This is in agreement with previous studies on rainbow trout, *Oncorhynchus mykiss* (Buyukcapar, Yanar, & Yanar, Y. 2007 and Hakan Yanar & Yanar, 2007). However, in Atlantic salmon, *Salmo salar* the whole-body lipid content was significantly higher in fish fed diets with carotenoids supplementation compared to the control diet (Christiansen and Torrissen, 1996). Similar feeding behavior was found in Tibbetts *et al*. (2010), where feeding with krill meal on cod *Gadus morhua*

and Atlantic halibut *Hippoglossus hippoglossus* increased feed intake, even at higher levels of digestible energy and protein. However, in white shrimp, *Litopenaeus vannamei* (Goytortúa-Bores, Civera-Cerecedo, Rocha-Meza & Green-Yee, 2006) and red porgy, *Pagrus pagrus* (Kalinowski *et al*., 2007), higher whole-body crude protein, crude lipid and dry matter were found in fish fed with CM diet compared to that fed the control diet. Moreover, CM has the role of meal attractant attributed to the soluble protein fraction content, preserved in meals with the adequate processing. Furthermore, CM contains certain soluble nutrients and a non-protein component, which might play a role in better dietary protein utilization, which is protein-sparing effect palatability and attractant diet effects (Yacoob and Browman, 2007).

Total body carotenoids content was enhanced by the addition of both natural and synthetic carotenoid sources. The highest body carotenoid concentration was observed in fish fed with AX0.1, followed by ML0.3 and CM15 diets. In this present study supported the previous finding demonstrating the role of carotenoids in pigmentation of fish (Armenta and Guerrero-Lagarreta, 2009). Other studies used an approach to employ ML in diets for rainbow trout, *Onchorhynchus mykiss* (Yanar, Buyukcapar, Yanar, & Gocer, (2007), red swordtail**,** *Xiphophorus helleri* (Ezhil *et al*., 2008), snow Trout, *Schizothorax richardsoni* (Jha, Sarma, & Qureshi, 2012). Queen loach, *Botia dario* (Gogoi, Mandal, & Patel, 2018) and koi carp, *Cyprinus carpio* (Swian *et al*., 2014) to obtain the acceptable body carotenoid concentration with extremely positive results. However, Laining, Trismawanti, Kamaruddin, & Makmur, (2017) reported that dietary carotenoid involved in the pigmentation of tiger shrimp, *Penaeus monodon* and improved the total carotenoids content in the whole body from 42.8 to 55.8 µg g^{-1} carotenoid. Pham, Byun, Kim, & Lee, (2014) also found that the total body carotenoids content of juvenile olive flounder, *Paralichthys olivaceus* were improved by AX supplementation.

High content of poly unsaturated fatty acids (PUFA) in marine fish makes them susceptible to oxidative stress, so it is important to add antioxidant nutrients in fish diets (Betancor *et al*., 2012). Previous studies have demonstrated the supplementation of carotenoids can significantly enhance the anti-oxidative capacity of fish and shrimp, thereby increasing their stress resistance and health (Li *et al*., 2014; Niu *et al*., 2014; Pham *et al*., 2014). However, dietary carotenoids could increase the liver antioxidant enzyme activities for Pacific white shrimp, *Litopenaeus vannamei* (Zhang, Liu, Tian, Yang, Liang & Yue, 2013), golden pompano, *Trachinotus ovatus* (Xie *et al*., 2017) and European seabass, *Dicentrarchus labrax* (Sallam *et al*., 2017). Diets supplemented with carotenoids enhanced antioxidant capacity may protect the organism from oxidative damage and reduce lipid hydroperoxides (Liu *et al*., 2010). Moreover, the improved effect of carotenoids regarding antioxidant capacity is in accordance with the results of Pan *et al*. (2010), that carotenoids alleviate oxidative damage and inactive free radical production due to cellular metabolism and various stressors. Carotenoid present in the diet as antioxidants can delay lipid peroxidation by inhibiting the initiation or propagation phase of oxidizing chain reactions by scavenging the free radicals (Shahidi and Zhong 2010). Meanwhile, the liver TBARs content was also significantly decreased in fish fed with higher dietary carotenoids than in those fed with lower ones. Similar results were found in olive flounder (Pham *et al*., 2014) and large yellow croaker, *Larimichthys crocea* (Yi *et al*., 2018), in which fish fed the carotenoid diets had lower TBARs content compared to that fed the control diet.

As was known, the environmental stresses such as thermal and osmotic stress tests have often been used as a final indicator of fish health status after feeding trials (Jin, *et al*., 2013; Cheng, *et al*., 2015). In the present study, increasing in dietary supplementation and body carotenoids significantly increased the survival rate of European seabass under physical stresses. This was in line with studies by Pan *et al*. (2011), who found dietary AX supplement increased the resistance and improved the survival rate of characins, *Hyphessobrycon eques* to ammonia stress. However, the improvement of survival rate against ammonia stress for tiger prawn, *Penaeus monodon* associated with an increase in dietary and body astaxanthin (Pan *et al*. 2003). A previous study has suggested that the inclusion of dietary 80-320 mg kg^{-1} astaxanthin can make a positive effect on immune and antioxidation capabilities and enhance resistance against high temperature stress of puffer fish, *Takifugu obscurus* (Cheng, *et al*., 2017). Moreover, shrimp (*Litopenaeus vannamei*) fed 80 mg kg^{-1} AX for 6 weeks showed significantly higher osmoregulatory capacity and survival than control diet after salinity was reduced from 35 to 3 gL^{-1} (Flores, Diaz, Medina, Re, & Licea, 2007). In the present study, it was clear that fish fed natural carotenoids enhance the tolerance and then the survivability of European seabass. These results indicated that natural carotenoids could improve the specific and non-specific immunity of fish. Thus, Yu *et al*. (2018) reported that natural carotenoids can significantly improve the survival rate and enhance disease resistance of Coral trout, *Plectropomus leopardus* challenged with *Vibrio harveyi*. Similarly,

Arous, El Bermawi, Shaltout, & Essa (2014) also indicated that natural carotenoids such as *Dunaliella salina* extract, crayfish meal and squilla meal has more impact on stress response of red tilapia, *Oreochromis Spp.*

Conclusion, in this study, supplementation of dietary natural carotenoid sources, such as marigold petals meal or crab-waste meal can improve the growth, antioxidant enzymes activities, stress resistance and total body carotenoids of sea bass. In addition, natural carotenoids such as marigold petals meal or crab-waste meal due to their lower cost and availability can be suggested as alternatives to the synthetic astaxanthin. According to results of the study, effective doses for the marigold petal meal and crab waste meal are 0.2 g kg^{-1} and 10 g kg^{-1} in sea bass diet, respectively.

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